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# Comparative aspects of the control of posture and locomotion in the spider crab *Libinia emarginata*

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COMPARATIVE ASPECTS OF THE CONTROL OF POSTURE AND LOCOMOTION IN  
THE SPIDER CRAB *LIBINIA EMARGINATA*

A Dissertation

Submitted to the Graduate Faculty of  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

The Department of Biological Sciences

by  
Andrés Gabriel Vidal Gadea  
B.S. University of Victoria, 2003  
May 2008

*For Elsa and Roméo*

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## ABSTRACT

The study of pedestrian locomotion in crustaceans has largely focused on forward walking macrurans, or sideway walking brachyurans. The spider crab, *Libinia emarginata* is a brachyuran that, unlike its close relatives, preferentially walks forward. The phylogenetic position, behavioral preference, and amenability to experimental techniques make spider crabs an attractive model for comparative studies of crustacean locomotion. This dissertation looks at the neuroethology of forward walking in *L. emarginata*. I described the skeletal, muscular, and neural anatomy of the walking machinery of *L. emarginata* and found adaptations at each level that reflect its walking preference. The ranges of motion of leg joints aiding in forward locomotion were larger for spider crabs than for sideway walking crabs. The leg segments housing the musculature moving these joints were also larger. The proximal leg musculature consists of multiple muscle heads that can be activated independently during locomotion. The motor neurons innervating this musculature exhibited features of distantly related species that walk forward. Unlike many brachyurans, spider crabs use all ten legs during walking. Kinematic characterization of forward walking in *L. emarginata* showed that anterior and posterior limbs perform different functions during walking. Cross-correlation analysis among the leg joints of spider crabs revealed that distant joints have stronger coupling than adjacent ones.

Neuroethology studies of pedestrian locomotion use multiple approaches. In order to understand how adaptive behavior is produced, it is necessary to study how the neural, muscular, and skeletal systems of an organism interact during its performance.

**CHAPTER 1**  
**INTRODUCTION**



## INTRODUCTION

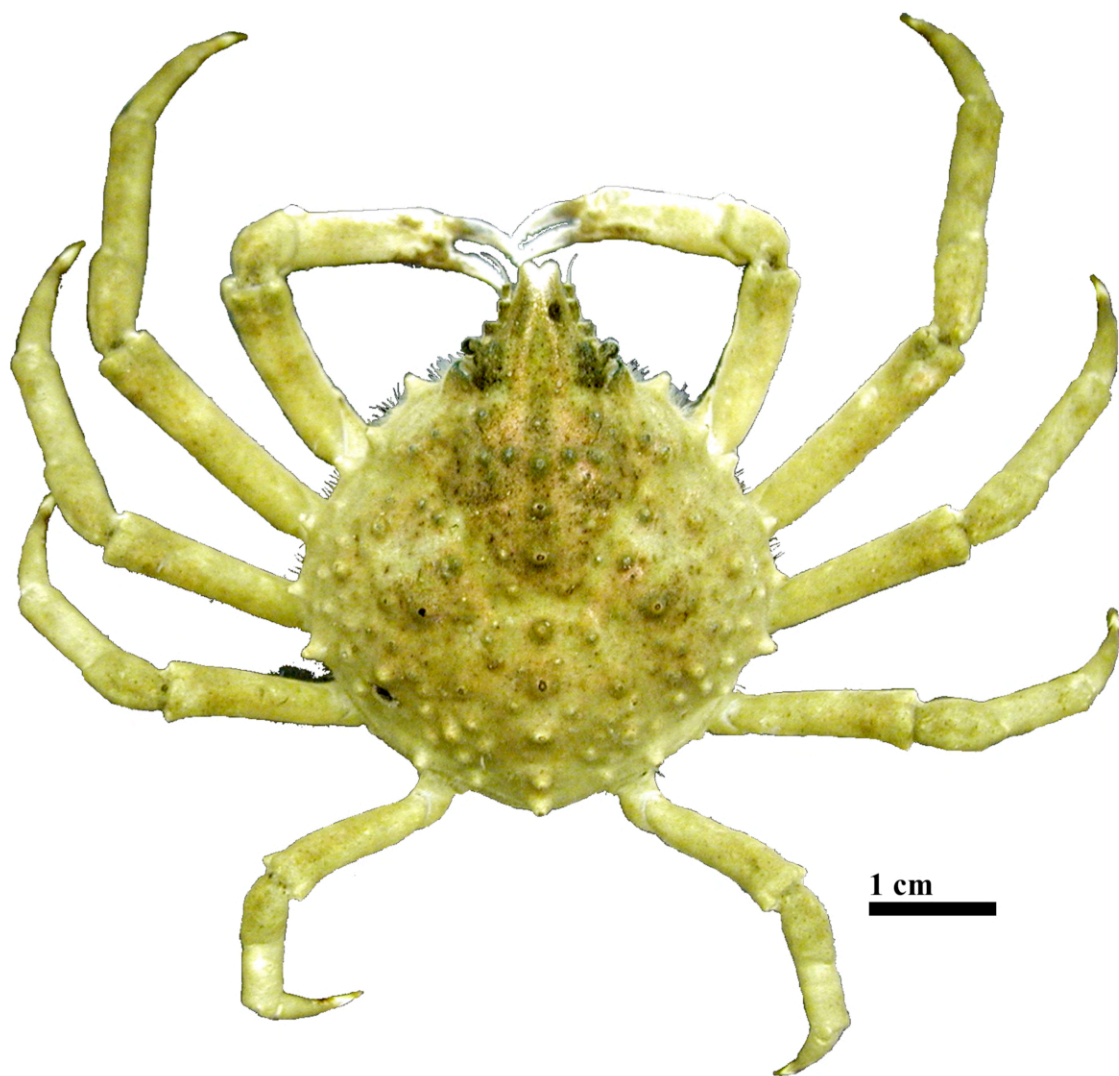
### **Crustacean Locomotion**

For many decades decapod crustaceans have served as model systems in neurobiology. The accessibility of their nervous systems, their more economical use of neuronal elements, and the wide range of interesting and relevant behaviors they are capable of producing are all reasons for their enduring appeal (Wiese, 2002).

The portly spider crab, *Libinia emarginata* (Fig 1.1) is a brachyuran (Majoidea, Decapoda) that walks preferentially in the forward direction about 80% of the time (Schreiner, 2004). As majoideans, spider crabs belong to the group thought to first have developed the crab form from lobster-like ancestors (Rice, 1983; Morrison et al, 2002). This makes *L. emarginata* potentially an interesting organism for comparative studies of brachyuran phylogeny.

### **Walking Behavior**

Studies of pedestrian locomotion in crustaceans can be divided into those looking at the performance of the behavior in freely walking animals (Ayers and Clarac, 1978; Barnes, 1975; Hui, 1992; Macmillan, 1975; Martinez et al., 1998; Sleinis and Silvey, 1980), and those studying the patterns of activity from isolated preparations (fictive walking, Hill and Cattaert, 2008; Sillar et al., 1987). Typically, the former will provide physical descriptions of the movements of joints and limbs (kinematics) (e.g. Jamon and Clarac, 1995), and sometimes also the concomitant activity of the musculature (Clarac et al., 1987). The latter consist of studies of isolated nervous systems and the production of walking-like patterns in the thoracic ganglia (Chrachri and Clarac, 1987; 1990; Leibrock et al., 1996). These two approaches are complimentary, but rarely have studies examined the link between the two by recording neural activity in freely behaving animals (e.g. Ballantyne and Rathmayer, 1981). A second way to link the two types of studies is to examine muscle activity and kinematics simultaneously, but this too was done infrequently



**Figure 1.1.** Dorsal view of the portly spider crab, *Libinia emarginata*.

in freely-behaving animals (but see Ayers and Clarac, 1978). Several groups have performed these studies in animals walking on treadmills (e.g. Ayers and Davis, 1977, Cruse et al., 1983), but there are potentially confounding issues introduced when animals are forced to entrain their behavior to the motion of the treadmill.

Studies of walking (reptantian) crustaceans have shown that sideways walking brachyurans primarily use alternating tetrapod gaits (Burrows and Hoyle, 1973; Barnes, 1975; Schreiner, 2004), although other gaits have also been described (Martinez et al., 1998). Forward walking crustaceans employ mainly metachronal gaits, and walk preferentially in the forward direction (Macmillan, 1975; Jamon and Clarac, 1995; Schreiner, 2004).

Patterns of rhythmic motor activity have been recorded in the isolated CNS of crustaceans (termed “fictive patterns”), and found to be correlated with the pattern of muscular activity observed during rhythmic locomotion. In the crayfish, rhythmic motor output was studied in the swimmeret system (Tschuluun et al., 2001; Mulloney, 2003) and the walking system (Chrachri and Clarac, 1990). Fictive locomotion lends itself to pharmacological manipulation, and it was shown that application of certain modulators can initiate or modify motor rhythms (Chrachri and Clarac, 1990). The major drawback of these kinds of studies is that in the absence of functional limbs attached to the preparation, it is difficult to unambiguously correlate this activity with the activity that would result if limbs were attached.

### **Environmental Challenges**

All walking animals need to overcome the challenges posed by their environment. Aquatic walkers need to overcome forces such as buoyancy and gravity that act in the vertical direction, and drag and acceleration reaction that occur in the horizontal direction (Martinez, 1996). Many times organisms need to operate in entirely different environments, such as on land and under water, and must be able to tune their behavior to their specific requirements (Clarac et al., 1987;

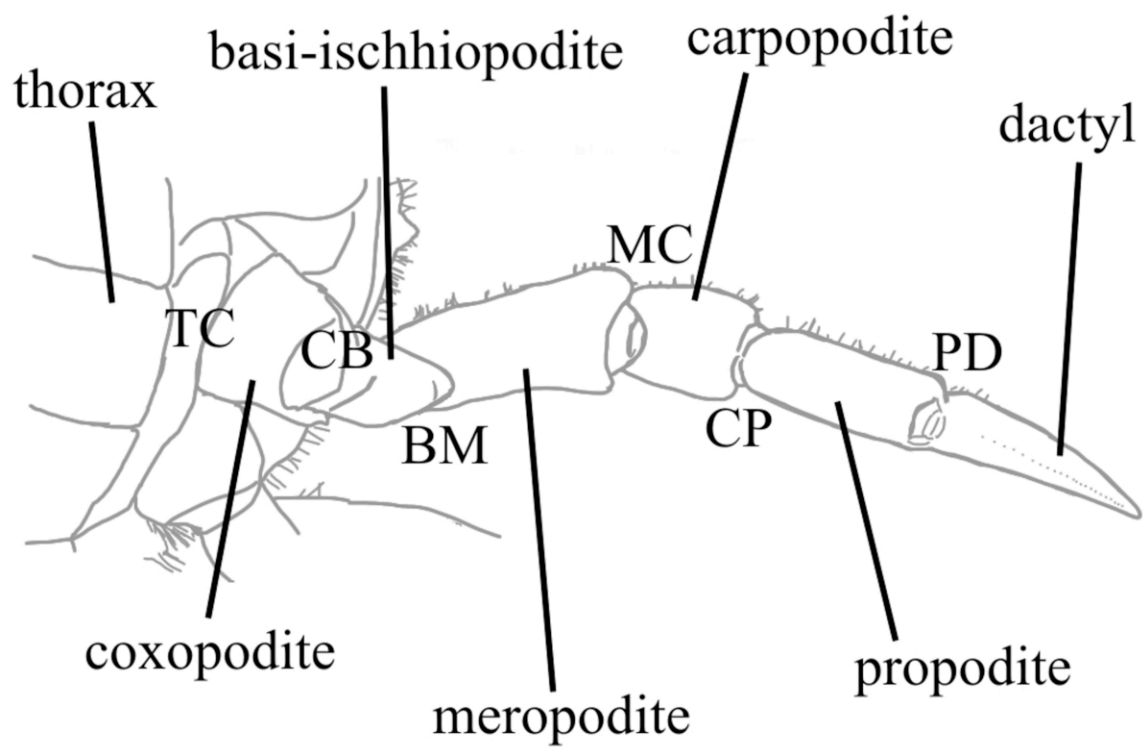
Schreiner, 2004). Some of the ways in which animals can cope with these environmental challenges include behavioral adaptations, such as changes in posture or gait (Martinez, 2001; Schreiner, 2004), and skeletal modifications, such as shell streamlining, to minimize the energy required to overcome drag (Blake, 1985).

### **Leg Musculature**

Crustacean legs have a basic plan consisting of seven segments (Fig 1.2). The main joints involved in locomotion are the thoraco-coxopodite joint (promoting and remoting the leg), the coxo-basipodite joint (adducting and abducting the leg), and the mero-carpopodite (extending and flexing) joint of the leg (Cattaert and Le Ray, 2001). Because the pereopod joints of crustaceans are bicondylar, they allow motion only in a single plane. By having these planes in successive joints perpendicular to each other, crustacean legs can articulate in any direction.

The musculature responsible for moving each of these segments consists of the following groups of antagonist muscles (from proximal to distal): promotor and remotor, levator and depressor, reducers, flexor and extensor, stretcher and bender, opener and closer muscles.

Crustacean muscle has been the subject of much research (for a review see Wiese et al., 1990). Physiologically, crustacean muscle fibers can be grouped into slow, intermediate, and fast types, typically co-occurring within the same muscle (Atwood, 1963; Fahrenbach, 1967; Fatt and Katz, 1953; Günzel et al., 1993; McDermott and Stephens, 1988; Tse et al., 1983). Slow fibers are characterized by a higher membrane resistance, capacitance, and myoplasmic resistance than fast muscle fibers (Raj and Cohen, 1964). In addition to the mentioned differences, crustacean fibers have several other unique features. Crustacean fibers can branch and anastomose, showing evidence of electrical continuity with neighbor fibers (Atwood, 1972).



**Figure 1.2.** The segments and joints composing the typical brachyuran leg.

## **Central Control of Pedestrian Locomotion**

The section of the crustacean nervous system that controls the motion of the walking appendages consists of the thoracic ganglia (Chrachri and Clarac, 1990). These are segmental neuromeres arranged sequentially in the thoracic portion of the ventral nerve chord. The ganglionic architecture of the thoracic ganglia has similar features across arthropod species (Averof and Akam, 1995). Comparisons between cockroaches (Davis, 1983), locust (Tyrer and Gregory, 1982), and the crayfish (Mulloney et al., 2003) revealed comparable numbers and location for the motor neuron somata, as well as similar dedicated integrating areas (neuropils) for the limbs of these species.

Although having a common layout (Mulloney and Hall, 2000), the thoracic ganglia of crustaceans are larger than their abdominal counterparts (Elson, 1996). This was attributed to an increase in neuropil size related to the greater complexity of movements controlled by these ganglia (Mulloney et al., 2003).

Despite the incredible diversity of behaviors produced by the limbs (Burrows and Hoyle, 1973; Faulkes and Paul, 1997a; Macmillan, 1975; Martinez et al., 1998; Wood, 1995), the numbers, location, and morphology of motor neurons controlling the pereopods of decapod crustaceans appear to be fairly constant across species (Fig 1.3; Wiersma and Ripley, 1952). The number of motor neurons innervating a single leg was estimated between 51 and 81, with most of the variability coming from the two most proximal joints (Faulkes and Paul, 1997b).

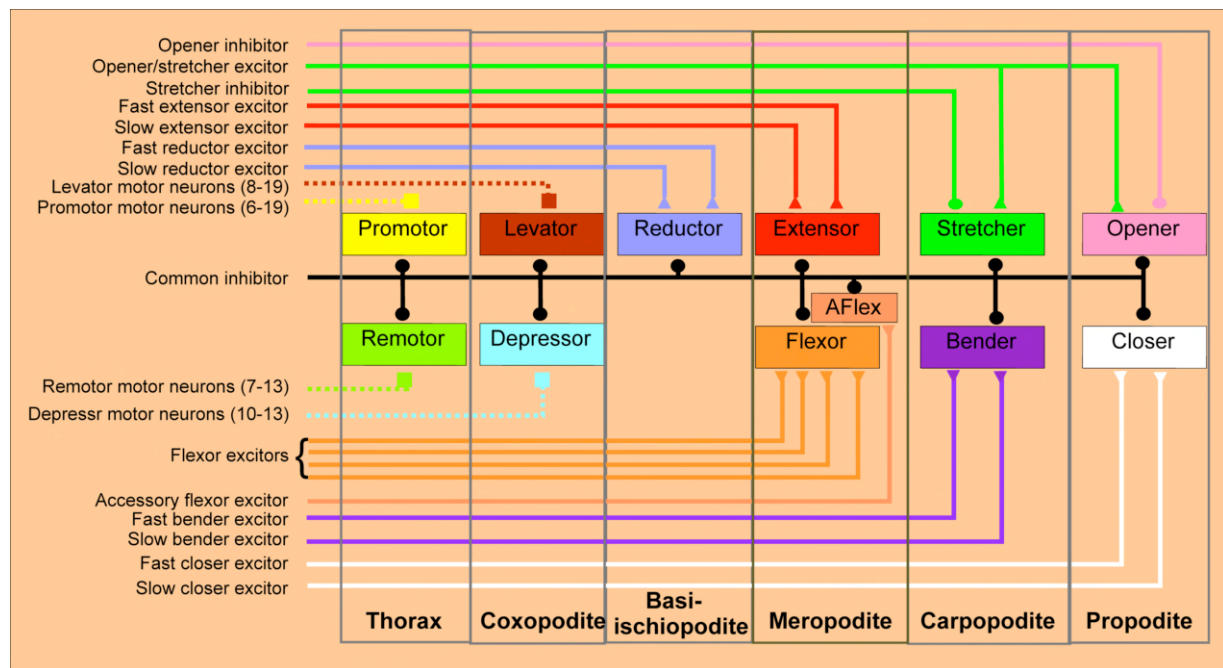
Within the ganglia, motor neuron somata are segregated by functional groups (Mulloney and Hall, 2000). For example, motor neurons innervating the promotor muscles are located in the anterior medial margin of the ganglia, while those innervating the levator musculature are found distally adjacent to these (Chrachri and Clarac, 1989). Lateral neuropils are greatly enlarged in

the thoracic ganglia of crayfish (Elson, 1996) and tend to invade the proximal roots of nerves exiting the ganglion in spider crabs (personal observation).

Crustacean motor neurons interact with each other to create highly efficient patterns of activity. Some motor neurons innervating the same muscle are electrically coupled (Chrachri and Clarac, 1989). Also, the presence of inhibitory motor neurons allows crustaceans to selectively activate a subset of fibers within a muscle (Ballantyne and Rathmayer, 1981). One of the most studied motor neurons in the arthropod walking circuit remains the common inhibitor neuron (Wiens and Wolf, 1993). This neuron innervates every muscle in the walking leg of brachyurans (Rathmayer and Bévengut, 1986, Wiens et al., 1988) and was hypothesized to be responsible for inhibiting tonic muscle fibers during walking (Atwood and Bittner, 1971; Atwood, 1976; Rathmayer and Erxleben, 1983).

Interneurons are less approachable than motor neurons and it is therefore not surprising that we know far less about them than about sensory or motor cells. Nonetheless, two major classes of interneurons were implicated in crustacean pedestrian locomotion: those coordinating the activity of different pereopods (either ipsi- or contralaterally) and those coordinating the activity of different muscles within a given pereopod (Chrachri and Clarac, 1989). The intersegmental coordination required for the production of meaningful locomotor rhythms was also shown to rely on several types of interneurons (Tschuluun et al., 2001).

The CNS also employs a plethora of chemicals to start, modulate, and extinguish rhythmic behavior. The channel types associated with different muscle fibers were shown to be differentially modulated by the neuropeptides (e.g. DF<sub>2</sub> and proctolin, Rathmayer et al., 2002). Fictive swimming in crabs (Wood, 1995), and fictive walking in crayfish (Gill and Skorupski, 1999) can be controlled by neuromodulators including amines and peptides. The diversity and plasticity afforded by these modulating substances becomes apparent when their actions are



**Figure 1.3.** Innervation pattern of crustacean pereopods showing the musculature of the leg and the number of motor neurons that innervate them (after Wiens et al., 1988 and Faulkes and Paul, 1997b).



combined. In crabs, different combinations of proctolin and dopamine were implicated in the production of behaviors as disparate as sideway and backward swimming, as well as courtship behavior (Wood, 1995).

### **Peripheral Control of Locomotion**

The legs of decapod crustaceans have a wide variety of sensory receptors that relay information back to the CNS (Cattaert and Le Ray, 2001; Leibrock et al., 1996). The information they convey includes limb location (Bush, 1965; Dunn and Barnes, 1981), stress (Libersat et al., 1987a, b) and velocity (Le Ray et al., 1997) to name but a few. Unlike their motor counterparts (whose numbers range between 50 and 80), sensory neurons in the legs of crustaceans number in the thousands (Libersat et al., 1987). Following the crustacean trend towards multifunctionality, these receptors do much more than just transmit sensory information. They are involved in many reflex pathways and their contribution can be essential for the generation of motor output (Hartman et al., 1997). As an example, the stretch reflex in the crustacean leg has been studied for many years. Its principal role is to oppose unwanted motion of the limb by applying force in opposition to the one experienced (Bush and Cannone, 1973). These pathways often involve sensory neurons synapsing directly onto motor neurons (Le Ray and Cattaert, 1999).

Two of the most studied sensory receptors in the legs of crabs are the muscle receptor organs (MRO), and the chordotonal organs. The former encode information about muscle length and tension, while the latter encodes angular changes in leg position (Cattaert and Le Ray, 2001). These organs are modulated by various substances (Rossi-Durand, 1993), and are also subject to pre- and post-synaptic inhibition from motor neurons (Atwood et al., 1984) and other sensory organs (Bush et al., 1978).

## **The Study of Pedestrian Locomotion Using Spider Crabs**

Several qualities are unique to spider crabs that make them an attractive subject for the study of walking behavior. Spider crabs were shown to walk preferentially forward about 80% of the time (Schreiner, 2004), and also readily walk sideways, backward, and diagonally. This rich behavioral repertoire invites comparative studies on the production of locomotion within and between species. Their phylogenetic position (Table 1.1), probably basal to the brachyurans (Morrison et al., 2002) is also favorable for such studies. In addition, spider crabs are amenable to physiological recordings by having a thoracic nervous system that is accessible without disruption of the walking musculature (Chapter 2).

In this study I asked several questions pertaining to the production of locomotion in spider crabs. Because spider crabs walk forward most of the time, we were interested in finding skeletal adaptations that might reflect their walking preference. To answer this question, we measured and compared the ranges of motion and leg segments of spider crabs, shore crabs and crayfish (Chapter 2). The walking preference of spider crabs results in anterior limbs always leading the walk while posterior limbs always trail it. We were interested in describing the leg musculature of spider crabs to determine if there were differences between the musculature of anterior and posterior legs and between the leg musculature of spider crabs and sideways walking crustaceans (Chapter 3). Furthermore, we anatomically described the population of motor neurons that innervate the leg musculature and compared it to that of forward and sideways walking species (Chapter 4). We compared the kinematics of anterior and posterior legs during forward walking and recorded the electrical activity of the depressor muscle bundles of legs three and five to determine if differences in proximal muscle and motor neuron numbers translated into differences in muscle activation during the performance of free forward walks by spider crabs (Chapter 5).

**Table 1.1.** Phylogenetic relationship between *Libinia emarginata* and crustacean model systems used in neuroethology research (after Martin and Davis, 2001).

Phylum	Arthropoda			
Subphylum	Crustacean			
Class	Malacostraca			
Subclass	Eumalacostraca			
Superorder	Eucarida			
Order	Decapoda			
Suborder	Pleocyemata			
Infraorder	Brachyura			Astacidea
Section	Eubrachyura			
Subsection	Heterotremata		Thoracotremata	
Superfamily	Majoidea	Cancroidea	Ocypodoidea	Astacoidea
Family	Pisidae	Cancridae	Mictyridae	Cambaridae
Genus	Libinia	Carcinus	Mictyris	Procambarus
Species	<i>Libinia</i>	<i>Carcinus</i>	<i>Mictyris</i>	<i>Procambarus</i>
	<i>emarginata</i>	<i>maenas</i>	<i>platycheles</i>	<i>clarkii</i>
Common name	spider crab	shore crab	soldier crab	crayfish
Walking preference	forward	sideway	forward	forward



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## **CHAPTER 2**

### **SKELETAL ADAPTATIONS FOR FORWARDS AND SIDEWAYS WALKING IN THREE SPECIES OF DECAPOD CRUSTACEANS**

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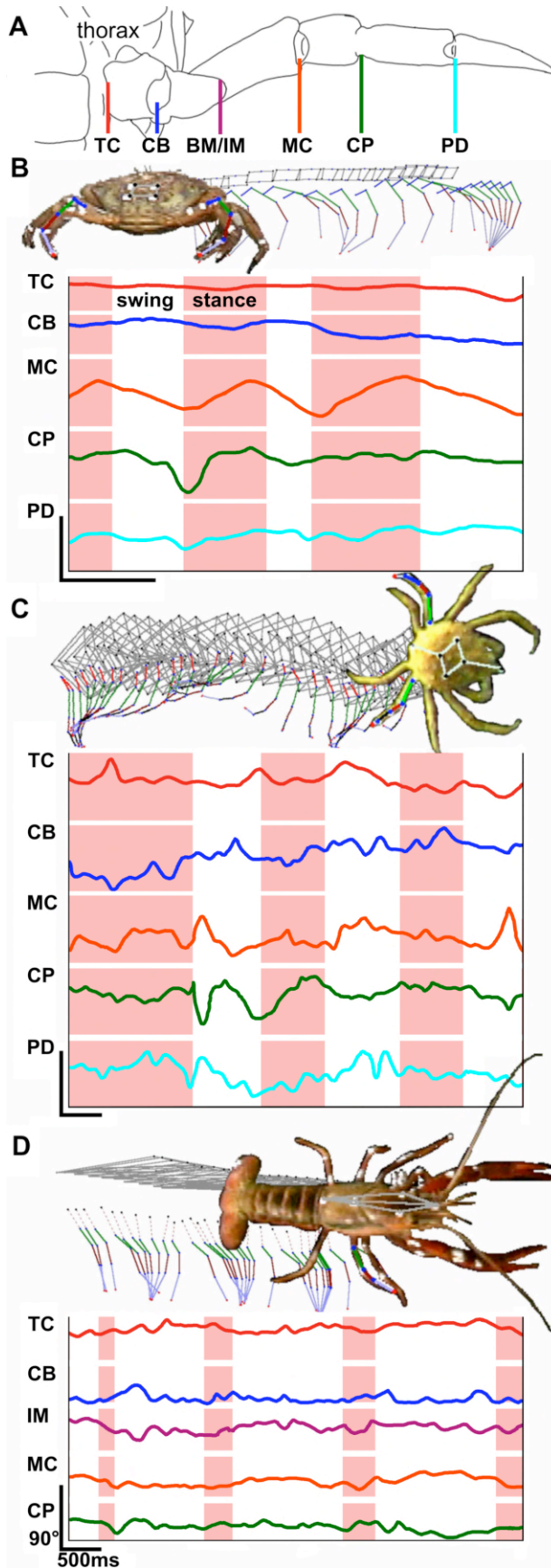
## INTRODUCTION

Most decapod crustaceans can walk in any direction. Even so, crayfish and lobsters walk forward most of the time, whereas crabs are remarkable in that many species walk primarily sideways (Blake 1985, Martinez 2001). From an evolutionary standpoint, crabs are believed to have first evolved from homoloid (lobster like) ancestors some 320 million years ago (Morrison et al., 2002). Molecular phylogenies suggest that the crab form arose independently no fewer than five times during evolution (Morrison et al., 2002). Whatever the advantage the crab form provides, it appears to be a one-way avenue, as no reversions to elongated bodies with extended abdomens are known.

Sideway and forward walking are the two main categories of progression in decapods, although intermediate (diagonal) walks were observed to occur in at least *M. platycheles* (Sleinis and Silvey, 1980), *Callinectes* (Weissburg et al. 2003), and *L. emarginata* crabs (personal observation).

Forward walking crustaceans usually employ metachronal gaits where waves of steps travel anteriorly (Macmillan 1975, Jamon and Clarac 1994). In sideway walking, the legs on one side of the animal lead the walk while the contralateral side trails them (Barnes 1975). Although most homoloid crustaceans walk primarily forward, and most crabs sideways, there are examples of crabs that walk primarily forward. The Australian soldier crab (*M. platycheles*) was shown to walk forward and uses metachronal gaits similar to those of macrurans (Sleinis and Silvey, 1980).

*Libinia emarginata* is a brachyuran of the family Majidae, proposed by Rise (1983), to be the first to have diverged from lobster-like ancestors. *L. emarginata* is an interesting system to study the neuroethology of walking in crabs, and it also possibly represents a transitional stage between forward walking homoloids and sideway walking crabs.



**Figure 2.1.** Walking behavior of *C. maenas*, *L. emarginata*, and *P. clarkii*. Plots showing the trajectories and angular excursion of the segments of the fourth pereopod during the typical walking behaviors of each species. The open blocks denote the swing phase of a step (no ground contact), while the pink blocks denote the stance phase (ground contact). For each animal the top trace shows the change in position over time of the points labeled in the legs and thorax during a walk. The bottom trace shows the angular excursion of the joints of the leg during the behavior shown (as calculated by Peak Motus). The joints are presented from proximal (top) to distal (bottom), and the scale bars are 90° and 500ms. **A)** *C. maenas*: typical angular excursions of the fourth leading leg (right, in this case) during sideways walking. **B)** *L. emarginata* walks forward with a metachronal gait, which is slower and has more irregular angular excursions. **C)** *P. clarkii* also employing a metachronal gait to walk forward.

Whether an animal walks forward or sideways, it will have to overcome several forces acting upon it. While some of these forces are independent of the walking preference (weight, lift, and buoyancy), other forces are not (drag, acceleration reaction) and need to be actively countered by a walking or even stationary animal (Martinez 1996, 2001, Martinez et al. 1998). Crabs walking forward will experience drag forces and acceleration reactions parallel to their longitudinal axis, while crabs walking sideways will experience drag forces that are perpendicular to their longitudinal axis. Furthermore, these forces are always present in a crab's life, and therefore the energy spent to overcome them must have an effect on an animal's fitness. We hypothesize that there will be skeletal adaptations of the morphology of a crustacean that act to minimize the energy required to operate under these circumstances. We propose that forward walking crabs and sideway walking crabs will have adaptations that reflect the preferred direction of locomotion.

The present study is the first of a series looking at the anatomy and physiology of *L. emarginata* as they pertain to its peculiar locomotor strategy. We looked for skeletal adaptations that might reflect *L. emarginata*'s preferred walking direction and compared them with the skeletal structures of the sideway walking crab *Carcinus maenas* and the forward walking crayfish, *Procambarus clarkii*. We chose these species based on the amount of research already conducted on the neuroethology of their walking behaviors (Bévengut et al., 1983; Brante and Hughes, 2001; Bush, 1962; Huxley, 1880; Jamon and Clarac, 1997; Martinez et al., 1998; Parsons, 2005; Pond, 1975).

## **MATERIALS AND METHODS**

### **Species Used**

*Libinia emarginata* and *Carcinus maenas* crabs were obtained from the Marine Resources Center of the Marine Biological Laboratories in Woods Hole, Massachusetts. Crabs

were kept at 20°C in artificial seawater until used. *Procambarus clarkii* were obtained from local suppliers and kept in fresh water until used.

### **Labeling Segments for Filming**

All animals were filmed standing in a 2 gallon aquarium on a felt substrate weighted down by a thin layer of sand (< 5 mm). Each animal was placed in the filming aquarium for one hour before recording in order to acclimate to their surroundings. Four cameras (three Canon GL-1 and a Canon ZR200) were used at different angles to capture video of the animals standing. Six animals of each species were filmed standing for 5-8 trials apiece. An individual trial was achieved by inducing the animal to walk, and then allowing it to come to rest of its own accord.

Points of interest were marked on each animal using Whiteout®. For each species, the carapace was marked at the tip of the rostrum, the widest distinguishable re-occurring points, and the most posterior location on the thorax (on midline). For *P.clarkii*, we labeled the posterior most point of the telson. For both crab species, the dorsal-most plane was defined by the four points formed by the intersection of the cervical and urogastric grooves and the brachial and cardiac grooves (*C.maenas*). In *L. emarginata*, the spines adjacent to the above named points were labeled. In crayfish, the dorsal midline was marked where it meets the cervical groove. All walking legs (2-5) were labeled similarly in all species, with the exception of the ischiopodites in leg 2 of *C.maenas*, which was visually obstructed from the cameras by the carapace. The remainder of the legs of the two crab species were labeled at the distal end of the segments: basis, merus, carpus, propodus, and dactyl. Due to morphological differences at the ischium, *P.clarkii* legs were marked at the distal end of the ischium, merus, carpus, and propodus. No measurements were made of the crayfish propodite-dactyl joint because of the small size of the dactyl segments. The first pair of legs, or the chelae, of *L. emarginata* and *P.clarkii* were

marked in the same manner, excluding the carpus. Line-of-sight obstruction by the carapace forced us to mark the chelae of *C. maenas* at the merus and dactyl only.

### **Data Analysis**

Statistical analyses were performed using SigmaStat 3.5. The angular excursion data were not normally distributed and were analyzed using non-parametric ranked ANOVA tests. Differences were considered significant at  $p < 0.05$ .

Video clips of each trial were imported into a 3D motion analysis system (Peak Motus) in order to obtain an averaged spatial representation of each animal. The raw coordinates of all the animals in each species were aligned in the same direction and averaged using Matlab in order to obtain caricatures of each species. Stance width was measured as the distance between dactyls of contralateral legs. Anterior-posterior leg arrangement was described as the longitudinal displacement of a leg's dactyl from the center of mass (CM, see Table 2.1 for abbreviations used in this study). All distal joint angles were directly measured and recorded. Proximal joint angles (TC and CB) were inferred by the horizontal displacement of the ischium from the insertion point of the leg on the thorax for the TC joint and the vertical displacement for the CB joint. Insertion points of each leg were found by measuring the spatial orientation of the leg's fulcrum and referencing it to the largest defined plane on the dorsal carapace. The walking trials were filmed at 30 frames/sec. All angles were calculated by Peak Motus.

### **Centers of Mass**

In all three species, the majority of the mass is contained in the thorax. We used the reaction board method (Reynolds and Lovett 1909) and an Ohaus Explorer digital scale to find the exact location of the CM in the thoraces. The masses for all the thoraces were recorded individually. The thoraces were then secured to the reaction board, which had one end resting on a pivoting point with the other end resting on the scale. The mass of the board alone was also

measured and the three values were used to calculate CM with the equation adapted from (Reynolds and Lovett 1909):

$$\sum T_a = 0 = (R) * (l) - (M_t) * (d) - M_b * (0.5l)$$

Where  $\sum T_a$  = Sum of all torques (N\*cm); R = Mass measured by the scale (g);  $l$  = Board length (cm);  $M_t$  = Thorax mass;  $d$  = CM-end of the board distance (cm);  $M_b$  = Board mass (g).

**Table 2.1.** Abbreviations used in this study.

<b>Abbreviations</b>	
<b>BI</b>	basis-ischium joint in <i>P.clarkii</i>
<b>BM</b>	basischium-merus joint in crabs
<b>CB</b>	coxa-basis joint
<b>CM</b>	center of mass
<b>CP</b>	carpus-propus joint
<b>MC</b>	merus-carpus joint
<b>PD</b>	propus-dactyl joint
<b>ROM</b>	range of motion
<b>T1-8</b>	thoracic somites 1 through 8
<b>TC</b>	thorax-coxa joint

Calculated CM for the thoraces were double-checked using the knife-edge method with a piece of plastic 1 mm in thickness (McKinon et al., 2004). The dorsal carapace was marked at the calculated position along the longitudinal axis. The CM for the thorax was defined as one-half the thickness of the thorax in the dorso-ventral plane measured at that location. The same method was used to find the CM in the abdomen of *P.clarkii*. All legs were removed at the thorax and masses were again recorded. The chelae were dissected into two parts: the distal end comprising the carpus, propodus, and dactyl, and the proximal end containing the coxa, basis, ischium, and merus. The segments were balanced on the lever to find the centers of mass. The remainder of the legs in *C. maenas* and *L. emarginata* were removed, and centers of mass were

found in a similar manner. The only difference in the procedure was an additional dissection at the propodite-dactyl joint. Peak Motus was used to combine segmental centers of mass to find the global CM for each individual. The legs of *P.clarkii* only contribute  $6.11 \pm 0.28\%$  of the total mass of an individual animal (compared to  $19.61 \pm 1.24\%$  in *L. emarginata*, and  $17.53 \pm 3.15\%$  in *C. maenas*). Because of the minimal contribution to the CM by each individual leg, we assumed that the local CM for that leg was located at 50% of the distance between the insertion point and dactyl.

### **Ranges of Motion of Leg and Leg Segment**

#### **Whole Leg Horizontal Ranges**

Animals were anaesthetized by cooling. The horizontal range of the entire leg was found by immobilizing the thorax in its resting position above graph paper. The legs were moved along the horizontal plane to the limit of their range and their path traced on the paper. The angle between the insertion point of the legs on the thorax and the points at either extreme of the range was measured.

#### **Individual Segment Ranges**

After anaesthetizing an animal, each segment was moved along its range of motion and the range recorded on the plane of motion using protractors. In order to compare animals of different species, we defined thorax size as:

$$s = \sqrt{(t_l * t_w)}$$

Where  $t_l$  = thorax length (cm);  $t_w$  = thorax width (cm).

#### **Segment Lengths**

We measured leg segment lengths ventrally using dial calipers. This was done using the distance between hinge points in consecutive segments.



## **Endophragmal Structure**

Animals were euthanized by cooling, and their dorsal carapace and organs were removed. The skeletons were immersed in 10% KOH in dH<sub>2</sub>O and incubated at 50°C for 14 days for *L. emarginata*, 7 days for *C. maenas*, and three days for *P. clarkii*. Because of the large amount of calcium in its skeleton, after KOH treatment the endophragm of *L. emarginata* was rinsed in dH<sub>2</sub>O and further treated with 10% HCl for 10 minutes to render the skeleton translucent. After incubation, the skeletons were placed in 5% Formalin and Janus green was applied to facilitate viewing.

Pictures of the processed skeletons were taken using a digital camera mounted on a dissecting microscope. Montage photographs were traced using Corel Photo-Paint® and were shaded by hand.

## **RESULTS**

### **Walking Behavior**

We refer to the legs by numbers 1 through 5 with leg 1 corresponding to thoracic somite 4 (T4), and leg 5 referring to thoracic somite 8 (T8). Previous work on *P. clarkii* showed the fourth pair of legs driving the walking pattern (Chrachri and Clarac, 1990). We measured the excursions of the segments comprising the fourth legs during a typical walking bout to determine which joints could be of particular interest for our study (Fig 2.1). *L. emarginata* walks more slowly than both *C. maenas* and *P. clarkii* (Fig 2.1B). During its forward walk, *L. emarginata* made more use of the TC, and CP joints than *C. maenas* while the MC angular excursion was smaller. *L. emarginata* also walks more jerkily than *C. maenas*. We should mention that although we recorded the kinematics of the fourth leg, the different legs of *L. emarginata* did not perform the same behavior during forward walking. The study of how each of the legs of *L. emarginata*

contributes to forward locomotion is beyond the scope of the present work and will be the focus of a future study.

### **Thorax Morphology**

For each species, the thorax is longer in the preferred direction of locomotion (Fig 2.2A). *P. clarkii* has the highest thorax length to width ratio at 1.77 ( $r^2=0.88$ ) followed by *L. emarginata* with a 1.09 ratio ( $r^2=0.95$ ). *C. maenas* is wider than long, with a thorax length to width ratio of 0.82 ( $r^2=0.85$ ). The thorax size (defined as the square root of thorax area) vs. body mass ratio has a similar slope for the three species: 0.33 ( $r^2 = 0.88$ ), 0.28 ( $r^2 = 0.92$ ), and 0.29 ( $r^2 = 0.96$ ) for *P. clarkii*, *L. emarginata*, and *C. maenas*, respectively. This suggests that weight should cause the same load on crabs of comparable thorax size (Fig 2.2B).

Of the three species studied, *L. emarginata* has the heaviest and most calcified thorax (it took twice as long to dissolve the skeleton of *L. emarginata* than that of *C. maenas*). The thorax to body mass ratio was  $0.75 \pm 0.01$  for *L. emarginata*,  $0.65 \pm 0.06$  for *C. maenas*, and  $0.55 \pm 0.02$  for *P. clarkii*. The thorax of *P. clarkii* is laterally compressed (Fig 2.3C). Compared to the dorso-ventral compression of *C. maenas* (Fig 2.3A), *L. emarginata*'s thorax is intermediate in form in that it is neither laterally, nor dorso-ventrally compressed (Fig 2.3B). While *C. maenas* has a thorax that is widest dorsally, the thorax of *L. emarginata* is widest ventrally. The thorax of *P. clarkii* does not vary greatly in width between the dorsal and ventral surfaces.

### **Endophragmal Structure**

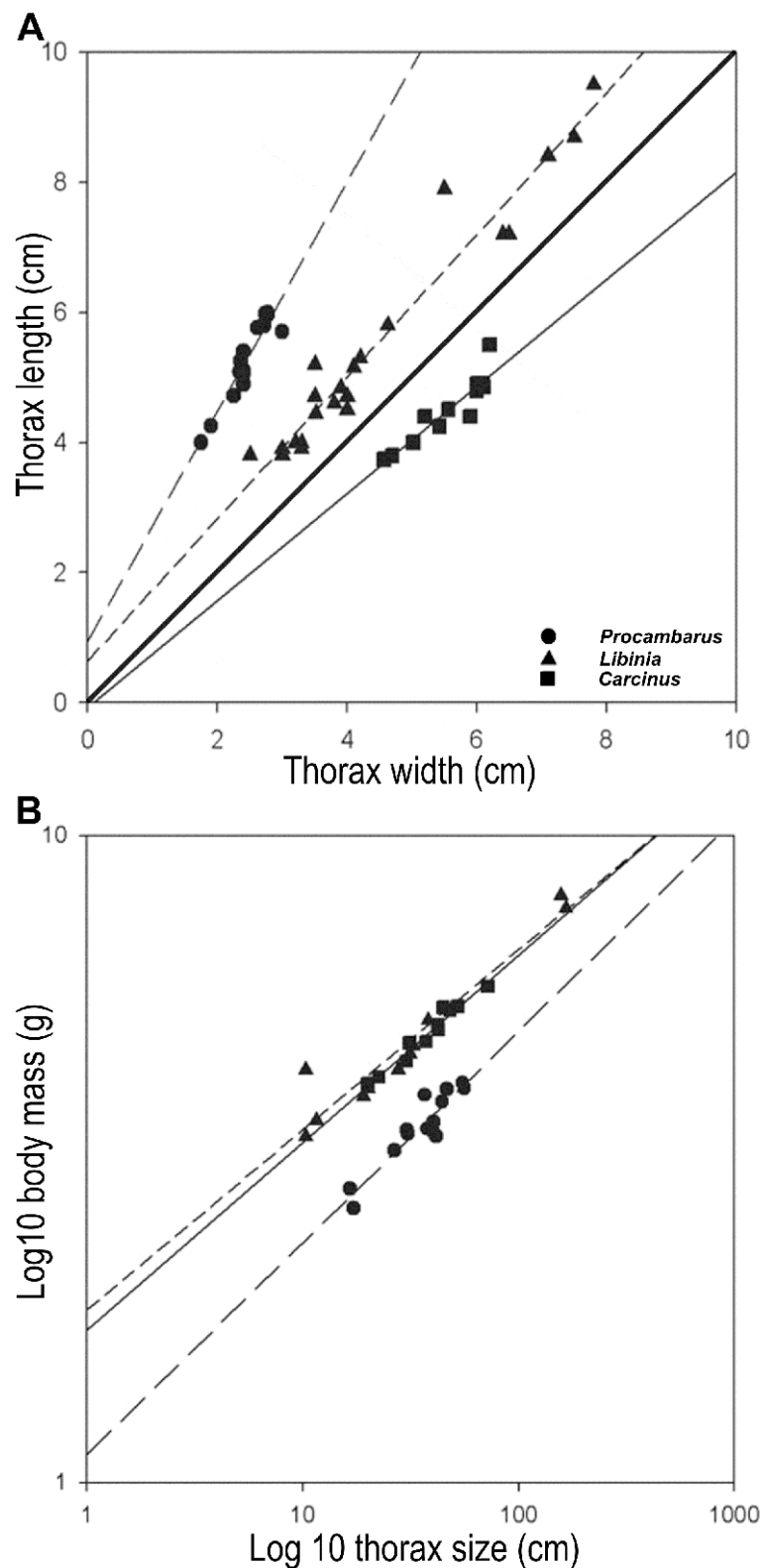
For the description of the endophragmal skeleton, we use the terminology of Pilgrim and Wiersma (1963). Removal of the dorsal carapace exposes the endophragmal skeleton housing the proximal leg musculature (Fig 2.3ii). The epimerites are the endophragmal structures that lie directly above the leg openings. They are segmentally arranged and have grooves between them from which the vertically invaginating endopleurites arise (Fig 2.3iiiiv). Each endopleurite gives

rise to an anterior and a posterior epimeral ridge (Fig 2.3iiid). These structures divide each endophragmal compartment into several cavities.

While the ventral compartments of each segment do not overlap with neighboring segments, the dorsal compartments overlap with posterior segments (Fig 2.3iii). Paralleling the radial arrangement of the legs around the thorax, the dorsal endophragmal units in *L. emarginata* lie more parallel to the longitudinal axis of the body than those in *C. maenas* (Fig 2.3Aii, iii, Bii, iii), increasing the possible length for the muscle they house. In both crabs the hinge points for the coxa are actually dorsal anterior, and ventral posterior so that the axis of the legs are rotated. This rotation is most pronounced in *L. emarginata*. *C. maenas* and *P. clarkii* differ from *L. emarginata* in having the dorsal endophragmal subunits more dorsally arranged (Fig 2.3iv, v). This allows them to accommodate the muscles despite the relatively narrow space that is available ventrally.

### **Leg Morphology**

*L. emarginata*'s legs are arranged radially around the thorax (Fig 2.3B). The legs of *L. emarginata* are all morphologically similar (Fig 2.4A) and decrease in size from anterior to posterior in a similar fashion to *P. clarkii* legs (Fig 2.4D). The leg segments are cylindrical in shape and heavily calcified. Sensory hairs are distributed along the entire surface of the legs of both *P. clarkii* and *L. emarginata* with higher concentrations at the joints. In addition to the sensilla covering the entire leg surface, *P. clarkii* has areas with a higher density of sensory hairs at the base of the fourth and fifth dactyl (Fig 2.4B) and the ventral edge of the propodite of the second and fourth pair of legs. The second legs of *P. clarkii* have chelae and are more flattened and sclerotized than the more posterior legs. The third legs of *P. clarkii* are also chelated. In contrast to the legs of *L. emarginata* and *P. clarkii*, the legs of *C. maenas* have sensory hairs only



**Figure 2.2.** Thorax shape and size. **A)** The thorax of each species is elongated in the direction of preferred locomotion. *P. clarkii* has the most elongated thorax in the anterior-posterior direction, *L. emarginata* has a slightly longer than wider thorax, while *C. maenas* is wider than it is longer. **B)** The ratio of thorax size to body mass is similar for *C. maenas* and *L. emarginata* and larger than for *P. clarkii*.

on the ventral surfaces of the second propodite and on the ventral and dorsal surface of the fifth carpopodites, propodites, and dactyls (Fig 2.4C). The legs of *C. maenas* and *P. clarkii* are laterally compressed. The legs of *C. maenas* slightly increase in size posteriorly (adj.  $r^2=0.929$ ), with the exception of the last pereopod which is specialized for swimming and is shorter and wider than the other legs. If the highly specialized second legs of *P. clarkii* and fifth legs of *C. maenas* are excluded, the length of the walking legs in all the three species is closely related to the size of the thorax ( $s$ ) (Fig 2.4D).

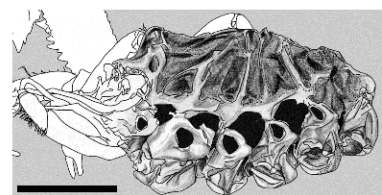
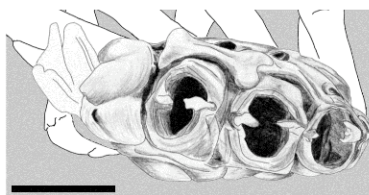
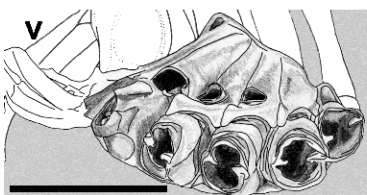
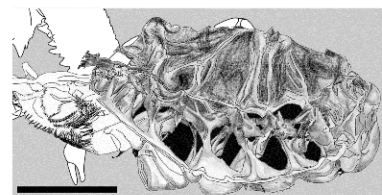
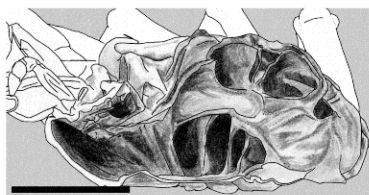
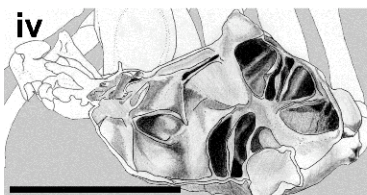
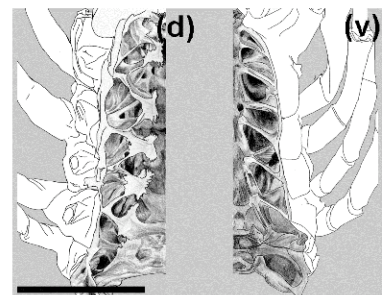
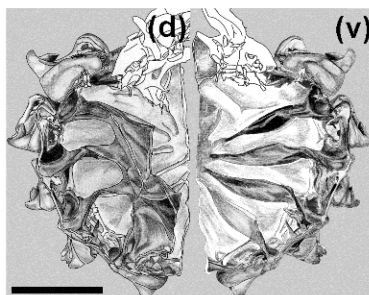
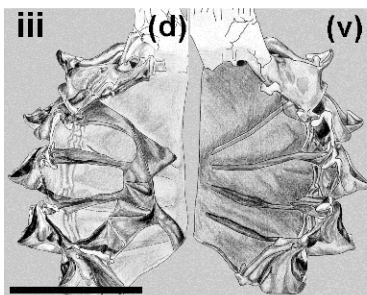
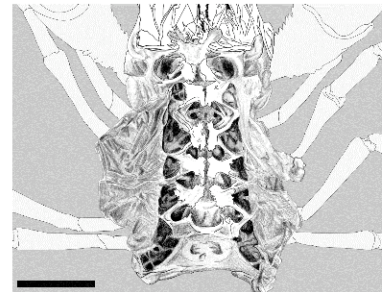
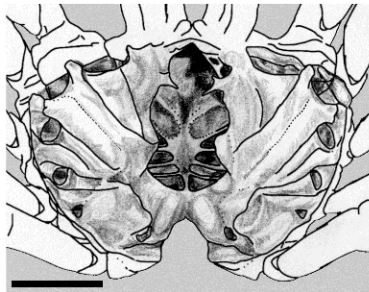
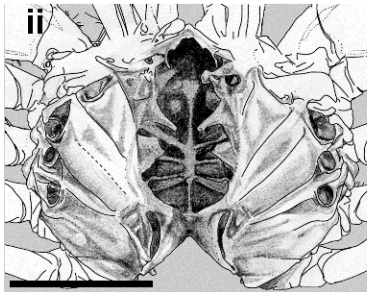
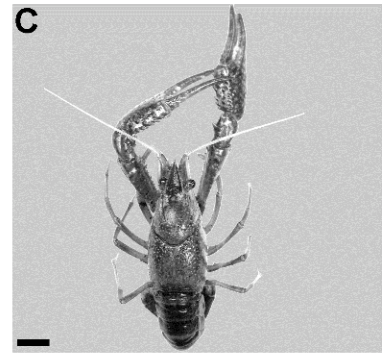
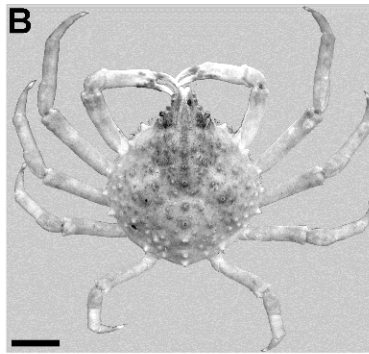
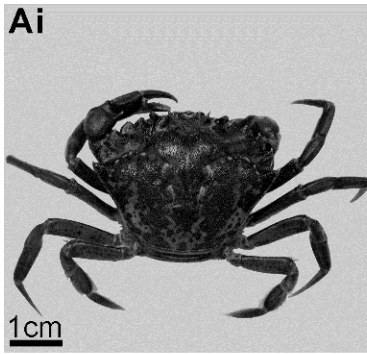
### **Combined Range of Motion for All Legs in the Anterior-Posterior Direction**

While the range of motion of the legs in the forward direction (horizontal plane) varies minimally within species, in *L. emarginata* it is more influenced by thorax size than in the other two species with adj.  $r^2$  between 0.21 and 0.45 (table 2). The combined horizontal range of motion for all legs is significantly larger for *P. clarkii* ( $312\pm11^\circ$ ,  $p<0.001$ ) than *L. emarginata* ( $250\pm17^\circ$ ) and is significantly smaller for *C. maenas* ( $223\pm5^\circ$ ,  $p<0.005$ ) (Fig 2.5B).

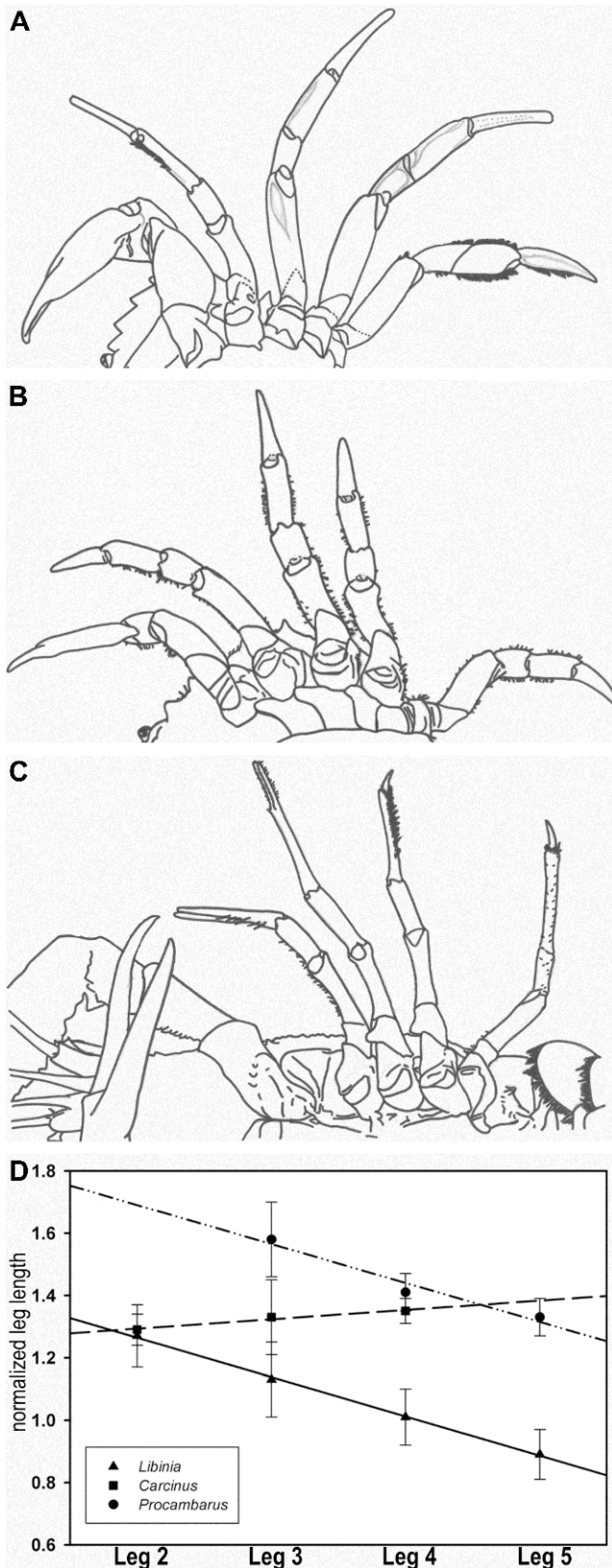
### **Ranges of Motion for Individual Leg Segments**

The joints of the legs of the crabs are uniplanar (with the exception of BM and CP in *L. emarginata*) and can be divided into those articulating forward-backward and side-to-side. The joints that move in the frontal plane are TC and CP. The joints that move the leg in the transverse plane are CB, MC, and PD. The plane of motion of BM in *L. emarginata* (and to a lesser extent in *C. maenas*) has its plane of motion diagonal to the lateral and frontal planes of the crab so that its movement results in a rotation of the longitudinal axis of the leg in the anterior direction. Joints used to propel the animals forward tended to have a larger mean range in *L. emarginata* and *P. clarkii* than in *C. maenas* (Table 2.3). The converse was true for joints moving the animals in the sideway direction. This trend was more pronounced for posterior legs (Fig 2.5C vs. D).

**Figure 2.3.** Endophragmal structure. **A)** Dorsal view of *C. maenas*, **(B)** *L. emarginata*, **(C)** and *P. clarkii*. **(ii)** For the three species, the thorax is longest in the direction of locomotion. Removal of carapace and digestion of soft tissue exposes the underlying endophragmal skeleton. **(iii)** Dorsal view of endophragmal structure after dissection of successive overlying layers. The proximal muscles for each leg are separated by endophragm into four groups by a horizontal dorsal rib (d), and a medial vertical rib (v). While the ventral ribs are aligned with the long axis of the legs, the dorsal ribs are arranged posteriorly. **(iii d)** In *L. emarginata* the dorsal endophragm is more oblique with respect to the midline than in *C. maenas*, or *P. clarkii*. **(iv)** Sagittal sections through the thoracic midline (left is anterior). The endophragm is taller in *C. maenas* **(iv A)** than in *L. emarginata* **(iv B)**, but shorter than in *P. clarkii* **(iv C)**. The convex nature of the ventral thorax of *C. maenas* **(iv A)** is apparent when compared with *L. emarginata* **(iv B)** and *P. clarkii* **(iv C)**. **(v)** Lateral view of endophragm after removal of ipsilateral legs. The legs of *C. maenas* **(v A)** are arranged less radially than the legs of *L. emarginata* **(v B)** but are not as parallel as those of *P. clarkii* **(v C)**.







**Figure 2.4.** Morphology of walking legs. **A)** Ventral view of the walking legs of *C. maenas*, **(B)** *L. emarginata*, and **(C)** *P. clarkii*. **A)** The legs of *C. maenas* are similar in morphology with the exception of the fifth leg, which is shorter and wider. The second and fifth legs are the only legs that have sensilla on their surface. **B)** The legs of *L. emarginata* are heavily calcified and have sensilla throughout their surface. They are not laterally compressed and are similar morphologically, appearing to differ only in length. In both *C. maenas* and *L. emarginata* the basipodite and ischiopodite are fused. **C)** *P. clarkii* legs make up much of the ventral thorax. They have unfused basis and ischium. The legs are laterally compressed with sensilla throughout, but like in *C. maenas* there are areas of dense sensilla density in the second and fourth leg. The second and third legs have chelae, with the second legs being shorter, and more sclerotized. **D)** Excluding the highly specialized second legs of *P. clarkii* and the fifth legs of *C. maenas*, the walking legs of *L. emarginata* and *P. clarkii* decrease posteriorly with the same slope. *C. maenas* legs, in contrast, increase in length posteriorly.



**Table 2.2.** Relationship between horizontal range of motion and thorax size.

<b>Adj.r<sup>2</sup> for horizontal ROM vs. thorax size</b>				
<b>Species</b>	<b>Leg 2</b>	<b>Leg 3</b>	<b>Leg 4</b>	<b>Leg 5</b>
<i>L. emarginata</i>	0.214	0.370	0.455	0.396
<i>C. maenas</i>	0.000	0.000	0.000	0.161
<i>P. clarkii</i>	0.023	0.025	0.000	0.000

### **Joints Moving in the Horizontal Plane**

#### **Thorax-Coxa Joint**

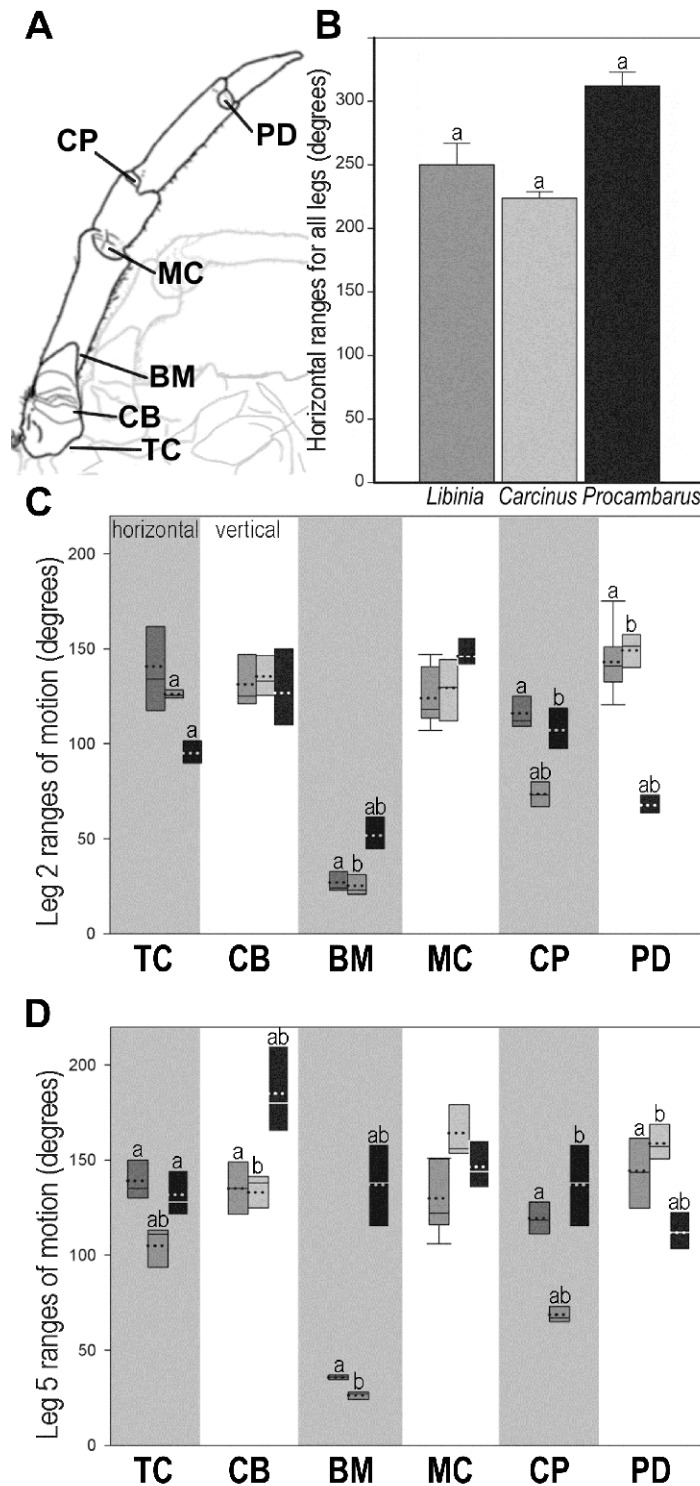
The range of motion of the TC joint for all legs combined was not significantly different between *L. emarginata* and *C. maenas* (n=46 each, see Table 2.3). The larger range of motion in the TC joint in *L. emarginata* than in *C. maenas* became more pronounced (and significant) for each posterior leg (Fig 2.5C, D). Both *L. emarginata* and *C. maenas* had a greater ( $p<0.001$ , and  $p<0.05$ , respectively) TC range of motion than *P. clarkii*.

**Table 2.3.** Ranges of motion for the joints of all the walking legs of the three species studied. Joint angles with the same superscript differ significantly.

		<i>P. clarkii</i>	<i>L. emarginata</i>	<i>C. maenas</i>
Horizontal plane joints	<b>TC</b>	110±26 <sup>o1,2</sup>	128±29 <sup>o1</sup>	123±24 <sup>o2</sup>
	<b>BM (BI)</b>	(69±30°) <sup>1,2</sup>	30±10 <sup>o1</sup>	26±6 <sup>o2</sup>
	<b>CP</b>	123±17 <sup>o1</sup>	97±26 <sup>o1</sup>	79±11 <sup>o1</sup>
Vertical plane joints	<b>CB</b>	155±33 <sup>o1,2</sup>	130±20 <sup>o1</sup>	138±15 <sup>o2</sup>
	<b>MC</b>	149±12 <sup>o1</sup>	144±18 <sup>o2</sup>	123±22 <sup>o1,2</sup>
	<b>PD</b>	86±22 <sup>o1,2</sup>	147±18 <sup>o1</sup>	151±11 <sup>o2</sup>

#### **Basis-Merus Joint**

*L. emarginata* and *C. maenas* had similar BM ranges. While in the crabs the BM joint rotates the leg forward, in *P. clarkii* the joint with equivalent action is the IM, which rotates



**Figure 2.5.** Leg ranges of motion for all the legs of the three species. **A)** The leg of each crab is composed of six joints, but *P. clarkii* has seven joints because its basis and ischium are not fused (not shown here). Each of the segments comprising the legs was measured. **B)** The combined range of motion in the horizontal plane for all legs is greatest for *P. clarkii*, and smallest for *C. maenas*. **(C, D)** Range of motion of each joint in leg 2 (C) and leg 5 (D). The boxes show the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles with the solid lines indicating the median and the dotted lines indicating the mean (n=10). Comparing the mean range of motion of individual joints reveals that animals tend to have a greater range of motion for joints articulating in their preferred direction of locomotion. This trend increased posteriorly as shown by the two extremes of legs 2, and legs 5. Bars with the same letter are significantly different. Solid lines, medians; dotted lines means. The data were analyzed using non-parametric ranked ANOVAs. Gray columns denote joints articulating in the horizontal plane, while white columns for joints moving in the vertical plane.

around a vertical axis and contributes considerably to forward locomotion. *P. clarkii* had a larger range of motion than *L. emarginata* or *C. maenas* ( $p < 0.001$ ).

### **Carpus-Propus Joint**

Although *P. clarkii* had the largest CP range of motion, in *L. emarginata* the CP range of motion was significantly larger ( $p < 0.0001$ ) than in *C. maenas*.

### **Joints Moving in the Vertical Plane**

#### **Coxa-Basis Joint**

Both *L. emarginata* and *C. maenas* ( $n=24$  each) had similar CB ranges of motion. *P. clarkii* ( $n=32$ ) had a larger CB range than either crab ( $p=0.001$ , and  $p=0.02$  respectively).

#### **Merus-Carpus Joint**

*P. clarkii* ( $n=32$ ) and *C. maenas* ( $n=23$ ) had largest ( $p < 0.001$ ) ranges of motion for the MC joint than *L. emarginata* crabs ( $n=37$ ).

#### **Propus-Dactyl Joint**

*L. emarginata* ( $n=36$ ) and *C. maenas* ( $n=22$ ) had similar ranges of PD motion. The dactyl of *P. clarkii* is greatly reduced and serves as a claw in the first two walking legs. Consequently, the range of motion was smaller for PD in *P. clarkii* than in the crabs.

### **Length of Leg Segments**

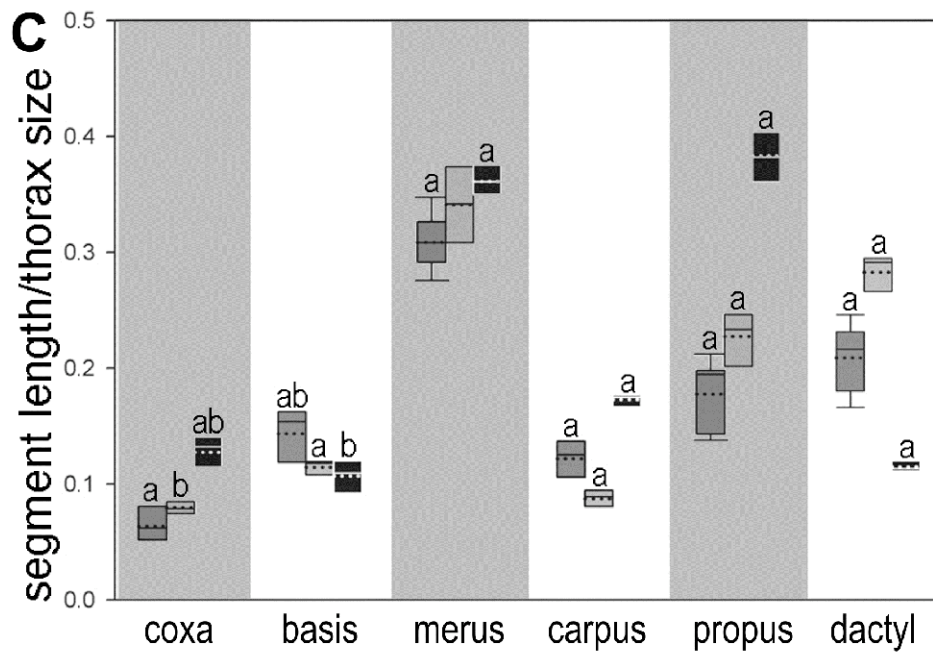
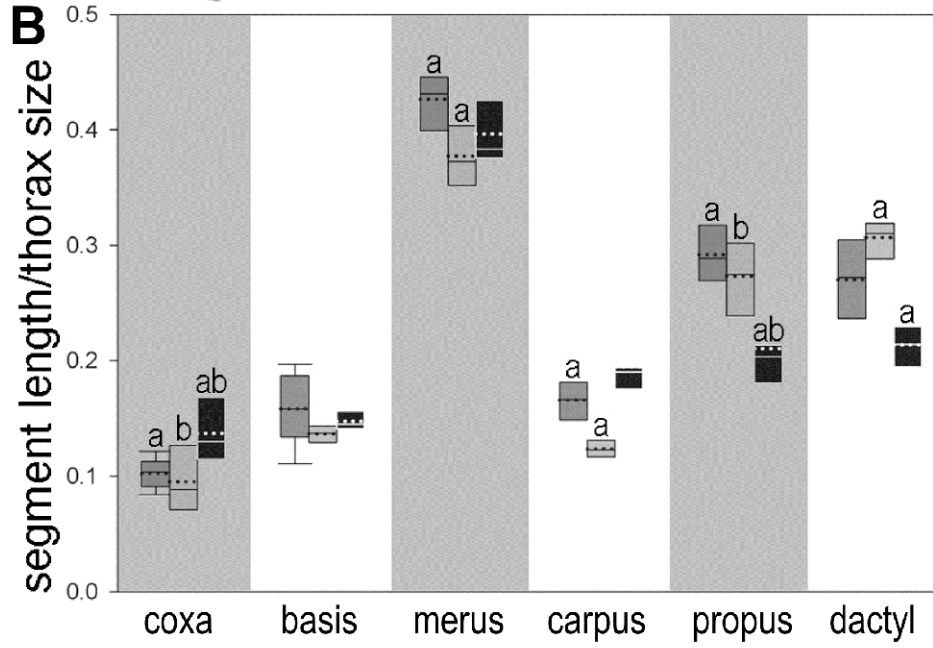
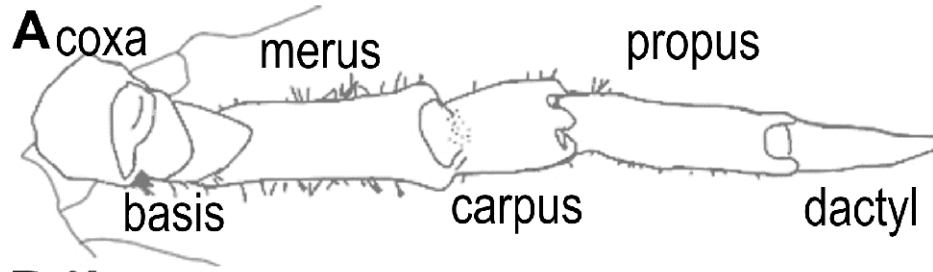
In the three species, the leg segments housing the musculature for a particular joint were longer for joints with larger ranges of motion (Fig 2.6). Paralleling the ranges of motion, the effect was smallest for the second legs (Fig 2.6B), and largest for the fifth legs (Fig 2.6C). Although the trend was altered by specialized legs (e.g. *P. clarkii* second propodite), in general the segments that housed the musculature moving a joint that had a greater range of motion in one species were longer than in species with a smaller range of motion. In order to compare segment lengths across species, we normalized the length of the segments to the thorax size.

Segments that house the musculature for a joint moving in the forward direction (basis and carpus) tended to be longer in *L. emarginata* ( $0.14 \pm 0.02$  and  $0.12 \pm 0.2$  respectively for leg 5) than in *C. maenas* ( $0.11 \pm 0.01$  and  $0.09 \pm 0.01$ ). The converse was also true of segments proximal to joints moving the leg in the sideways direction (merus and propus), where the sideways walking *C. maenas* had higher ( $0.34 \pm 0.03$  and  $0.23 \pm 0.02$  respectively for leg 5) segment length to thorax size ratios than *L. emarginata* ( $0.31 \pm 0.02$  and  $0.18 \pm 0.03$  respectively). Because the TC joint musculature is largely contained within the thorax and because the dactyl does not house any musculature, the coxa and the dactyl were not used in this comparison.

### **Leg Arrangement and Stance**

After comparing body and limb morphologies and ranges of motion, we looked for differences in joint use at rest. We videotaped animals standing motionless and generated average limb configurations, body postures, and calculated centers of mass (Fig 2.7A). The insertion points of the legs onto the thorax were determined and are shown in ventral and lateral aspects (Fig 2.7B). *C. maenas* and *P. clarkii* stood with their limbs in an elliptical pattern elongated in the preferred direction of movement (Fig 2.7C). *L. emarginata* maintained a more circular arrangement of the legs. Each animal stood with the legs arranged in an arc so that legs 3 and 4 had the largest width from the thorax compared to legs 2 and 5 (Fig 2.7D). *C. maenas* adopted a wider stance with leg length paralleling interleg width (compare figs 4D and 7D), whereas in *L. emarginata* the longest (L2) and shortest legs (L5) were similarly spaced. *P. clarkii* incorporates a wider stance in the fifth leg and a much shorter interleg distance between the specialized second legs. *P. clarkii* further differs from the other two species in having the CM between legs 4 and 5, and as a result only leg 5 is behind the CM of *P. clarkii*. The third leg pair of *P. clarkii* is positioned much closer to the second legs, suggesting that each pair of legs provides similar stability in the direction of the longitudinal axis. In the crabs the CM is between

**Figure 2.6.** Segments composing the crustacean leg. **A)** The legs of the crabs are composed of six segments (since the ischium and basis are fused), while in *P. clarkii* there are seven segments. **(B, C)** Box-whisker plots showing mean segment length, normalized to thorax size (s), for the second leg (B) and the fifth leg (C) of each of the three species tested (n=8 for each). There was a positive correlation between joints having increased ranges of motion, and the length of the segments proximal to these joints. In *C. maenas* segments immediately proximal to joints moving in the sideways direction were longer. *L. emarginata* and *P. clarkii* had longer segments proximal to joints that moved in the forward-backward direction. This trend increased posteriorly with leg 2 (**B**), and leg 5 (**C**) being the extremes. Boxes with the same letter are significantly different. These data were analyzed using two way ANOVAs. The boxes show the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup>, percentiles with the solid lines indicating the median and the dotted lines indicating the mean.



the third and fourth legs. Spacing between adjacent legs 3 and 4 is similar in both *L. emarginata* and *C. maenas*; however, both legs 3 and 4 are shifted anteriorly in *C. maenas* resulting in the CM being closer to legs 4.

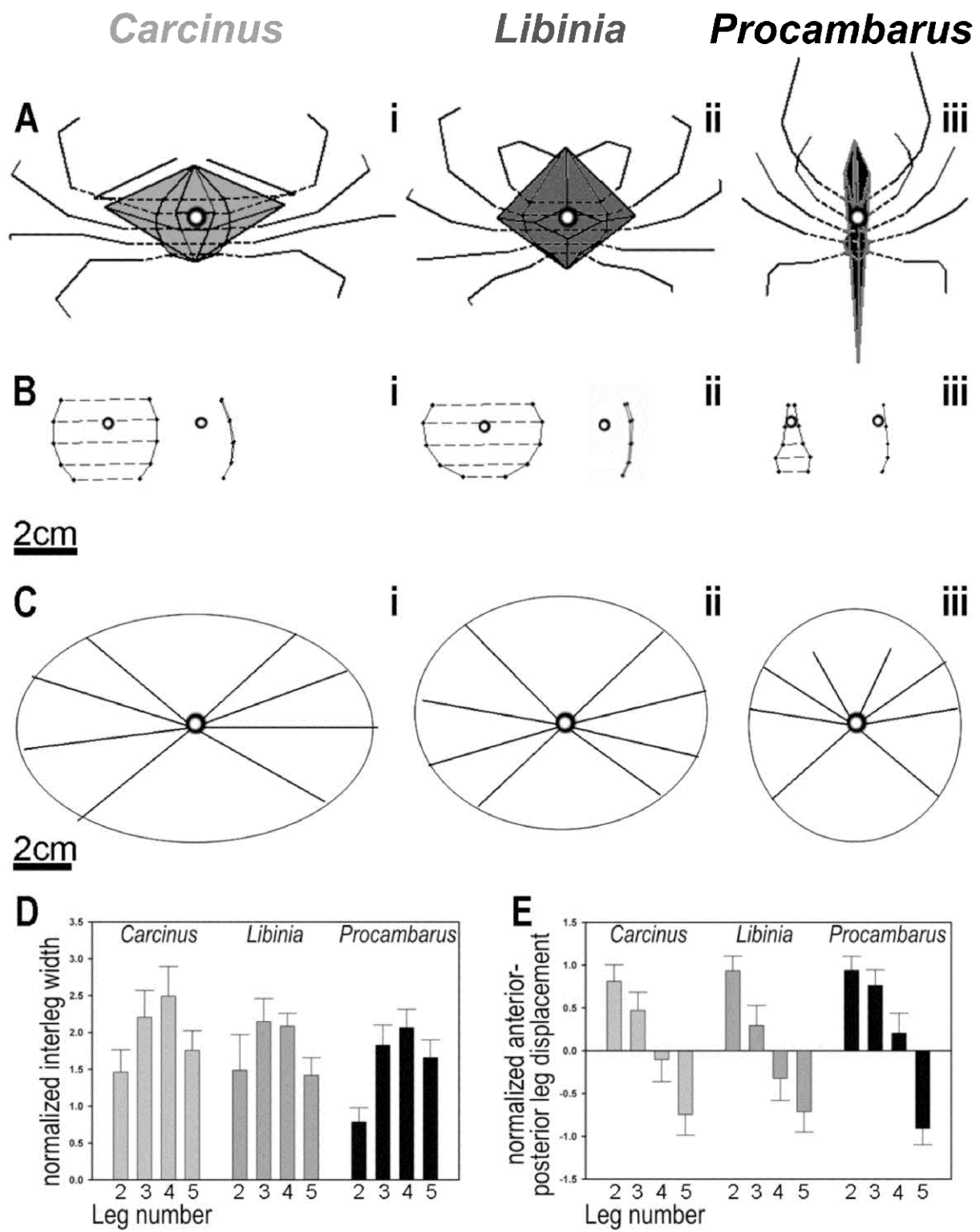
### **Joint Contribution to Stance**

We next investigated how each of the joints contributed to the position of each of the legs. In the brachyurans there was a marked difference between all angles across all legs that contribute to displacement in the vertical (Fig 2.8 patterned bars) and horizontal plane (solid bars). The majority of the horizontally displacing joints (TC, BM, CP) were much straighter, or closer to 180°, than the joints that contribute to motion in the vertical plane (CB, MC, PD). When compared to *P. clarkii*, the two vertical joints measured (CB & MC) were very different. The CB joint was very near 180° in all legs while the MC joint was the closest to perpendicular in all species. This suggests that the MC in crayfish generates and is exposed to different forces at rest than the CB joint. In the crab species, all three vertically moving joints appear to generate and be exposed to similar forces at rest. The CB angles also suggest that the limbs exit the thorax in a more horizontal plane in *P. clarkii* than in the crabs.

For the TC joint there were similarities between behaviorally similar animals. In *C. maenas*, all of the TC joint angles were >159° (L2= 159±9°; L3= 174±4°; L4= 175±3°; L5= 169±6°), which means that the legs were close to perpendicular to the longitudinal axis of the crabs. *L. emarginata*, however, had a wider range of TC angles. Legs 2 and 4 were 159±9° and 162±11°, while leg 3 exited the thorax with a large (straight) angle (172±5°). Conversely, leg 5 TC joint had an average angle of 145±11° directed posteriorly. In *P. clarkii*, all four TC joints had an angle of <162° (L2= 141±8°; L3= 151±8°; L4= 162±10°; L5= 162±9°). This suggests that in forward walking species the TC joint contributes more to the observed leg spread in the anterior-posterior direction (Fig 2.8A-C).

**Figure 2.7.** Three-dimensional caricatures of *C. maenas*, *L. emarginata*, and *P. clarkii* (dorsal views Ai-iii) based on averaged coordinates of five animals. The leg insertion points in the carapace are shown (B) in dorsal and lateral views (anterior is up). The placement of the dactyls around the CM forms ellipses elongated in the preferred direction of locomotion (Ci-iii). Centers of mass are shown by black and white circles. **D.** The interleg distance between paired legs normalized to thorax size,  $s$ . Each species has the largest spread between legs 3 and 4, with *C. maenas* having a wider stance than the two forward walkers. **E** graph of the overall leg displacement from the CM in the anterior-posterior direction. *C. maenas* and *P. clarkii* show less symmetry in legs three and four than does *L. emarginata*. Both brachyurans stand with their CM between dactyls 3 and 4, while in *P. clarkii* it lies between dactyls 4 and 5.





A closer look at the distal horizontally moving joints (BM and CP) across species revealed additional differences. In *C. maenas* these joints were at rest bent away from the CM, thus maximizing stability. In *L. emarginata* distal horizontally moving joints (CP) were always in a bent (forward) position, which is perhaps useful in forward walking since the joint would already be primed for action. In the posterior two legs of *P. clarkii*, most of the horizontal excursion can be attributed to the IM joint.

## **DISCUSSION**

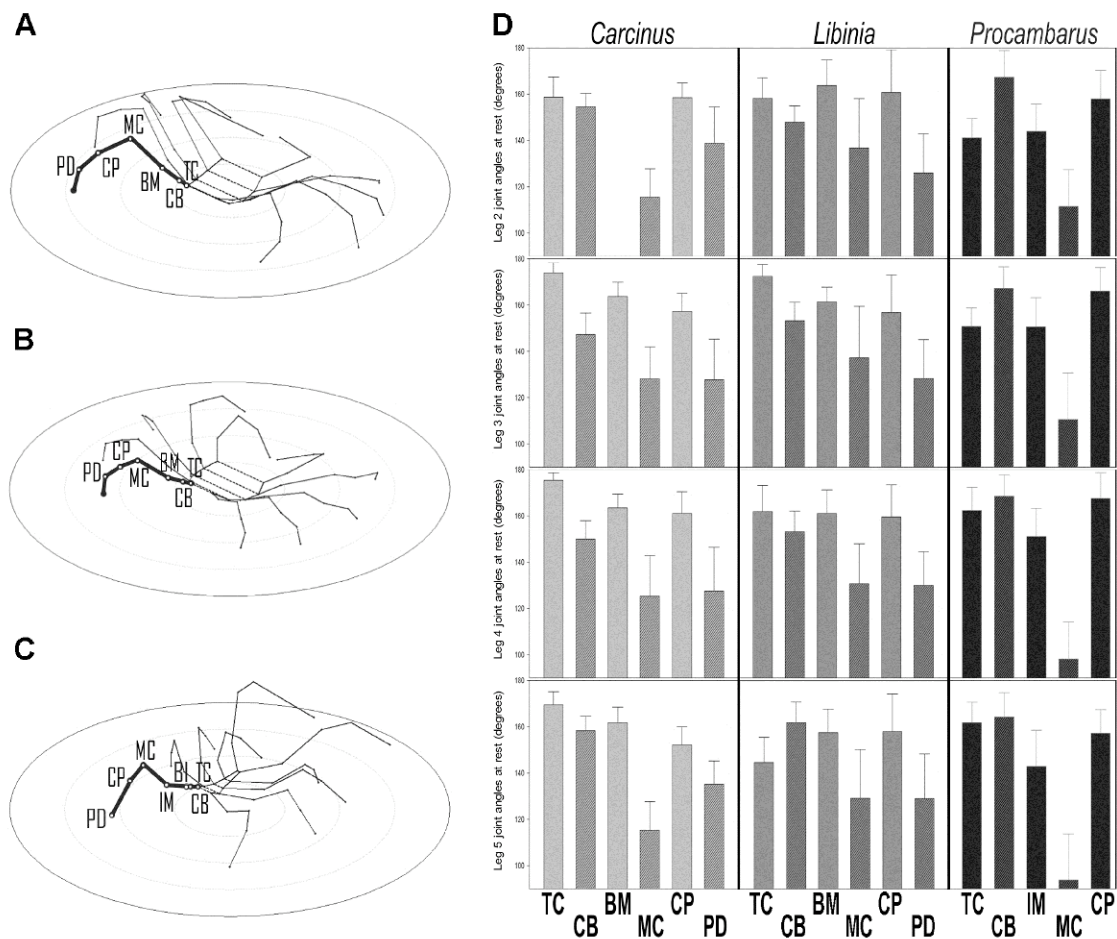
### **Walking Preference**

*L. emarginata* crabs walk preferentially in the forward direction (Schreiner, 2004) employing primarily metachronal gaits similar to those used by macrurans (Fig 2.1). Walking in the forward direction means that each leg is committed to either pull or push the center of mass, which is not the case for sideways walking crabs. This distinction means the limbs of *L. emarginata* could be specialized with front and hind limbs performing different functions (see Ritzmann et al., 2004). Our preliminary observations of musculature and the kinematics of walking in *L. emarginata* further support this view.

### **Thoracic Structure**

Although *L. emarginata* and *C. maenas* differ in walking strategies, skeletal adaptations, degree of calcification, and their thoracic size to mass ratios are similar. Therefore, they are exposed to similar forces along the vertical axis (Fig 2.2B).

All three species exhibited elongation in the preferred direction of locomotion. Previous studies on shell hydrodynamics in blue crabs revealed the optimal streamline of crab shells is in the transverse direction (Blake, 1985). The commitment to a particular direction of locomotion seems to be reflected in the degree of elongation exhibited (Fig 2.2A). *P. clarkii* walks mostly in



**Figure 2.8.** Comparison of the joint angles of the legs at rest. A, B, and C show *C. maenas*, *L. emarginata*, and *P. clarkii*, respectively, all with the dorsal carapace removed. Bar graphs D, E, and F give the joint angles for the corresponding species. Joints are displayed from most proximal (TC) on the far left of the independent axis to most distal on the right. In the two crab species, joints that move in the horizontal plane are straighter (closer to 180°) than those that bend in the vertical plane. The majority of the range of motion in the vertical plane in the legs of *P. clarkii* can be attributed to the MC joint.

the forward direction (Pond, 1975) and has the largest elongation (1.77 length/width ratio). *L. emarginata* walks primarily forward, but also sideways about 20% of the time (Schreiner, 2004), and it has a 1.09 length to width ratio. *C. maenas*, however, rarely walks forward and has a 0.82 ratio. This finding is supported by the only other forward walking crab found in the literature (*Mictyris platycheles*) where the thorax is also longer than it is wide (Sleinin and Silvey, 1980). Blake (1985) also studied a non-brachyuran king crab and concluded that its carapace was not adapted to reduce drag; however, he assumed sideway locomotion and did not test past 20° from the sideway direction, thereby potentially missing the preferred walking direction of this animal. Drag is likely a major factor in determining thorax morphology, since animals have to spend energy to overcome it every time they move. Work on chemotaxis showed that blue crabs will deviate from the most hydrodynamic posture only when food and slow flows co-occur (Weissburg et al., 2003).

### **Endophragmal Differences**

While the thorax of *C. maenas* is widest dorsally, the thorax of *L. emarginata* is widest ventrally and can accommodate longer proximal musculature (levators, depressors, promoters, remoters) than *C. maenas*. This is correlated with the large range of motion of its TC joint (Fig 2.1). In contrast to *L. emarginata*, both *C. maenas* and *P. clarkii* have their endophragmal compartments arranged more vertically (Fig 2.3). This result could be related to their ability to walk on land, since the increase in experienced weight would require stronger forces to support the weight of the animal on land, and thus the animals would benefit from having shorter muscles with more fibers in parallel. Because *L. emarginata* does not venture on land, its proximal musculature doesn't have to deal with increased weight, and it can afford to have the longer muscles required for the increased TC range observed during forward locomotion. The posterior arrangement of the epimerites in *L. emarginata* further increases the potential length

that the muscles can reach and lends support to the importance of the TC and CB joints in this behavior (Fig 2.3Bii). The ventral endophragm was also different in *L. emarginata* and *C. maenas*, with the posterior segments (4 and 5) having a much more anterior orientation in *L. emarginata*. This is a reflection of the radial arrangement of the legs of *L. emarginata* and increases the length of the compartment available for the ventral musculature (Fig 2.3iii v).

### **Leg Specializations**

The specialization of the legs of decapod crustaceans is not always as obvious as a pair of chelipeds. The three species we studied have additional specializations in their walking legs. *C. maenas* legs are laterally flattened and mostly devoid of sensilla except for the second and fifth legs. The sensilla in these areas were shown to be involved in chemotaxis in blue crabs (Keller et al., 2003). The last pair of legs of *C. maenas* is shorter, wider, and rotated more horizontally to perform swimming behavior (Fraser, 1974). *P. clarkii* legs are flattened and have sensilla throughout, but (like those of *C. maenas*) have areas of greater sensilla population in the second and fourth legs. This is consistent with Keller et al. (2003) who suggested that because of boundary layers, the distal segments are exposed to slower, more viscous water that facilitates the task of tracking odorants. In *P. clarkii*, unlike in *C. maenas*, the second and third pairs of legs have chelae. *P. clarkii* differs from both crabs in not having the basipodite and ischiopodite segments fused, so it has an additional joint. In *P. clarkii*, the plane of the basis-ischium joint rotates the longitudinal axis of the leg, while the ischiopodite-meropodite moves in the anterior-posterior direction.

*L. emarginata* exhibits the least morphological differences between legs. Sensilla cover the legs (and thorax) homogeneously without clearly specialized sensory areas. The largest difference between the legs of *L. emarginata* is the angle between the longitudinal axis of the legs and of the thorax. This difference in insertion angles between the legs results in the legs

being evenly spaced around the perimeter of the thorax and therefore having a wide horizontal range of movement without contacting adjacent legs. Because each pair of legs inserts at a different angle, the action performed by analogous muscles in different legs will not be equivalent.

### **Ranges of Motion**

When we compared the combined angular displacement in the horizontal plane (forward direction), we observed that *P. clarkii* had the largest range of motion followed by *L. emarginata*, and lastly *C. maenas* (Fig 2.5B). Thus, the animals have a maximal range of motion in the preferred walking orientation. We next wanted to find out if this was correlated with the radial arrangement of the legs in *L. emarginata* (the legs of *P. clarkii* are the least radial of the three species) or with increased ranges of motion in the segments that move the leg in the anterior-posterior direction. We found that joints moving the legs in the forward direction had a larger range of motion in *P. clarkii* and *L. emarginata* than in *C. maenas* and the opposite was true for joints moving the leg in the sideways direction (Fig 2.5C, D). The difference increased for posterior pereopods (Fig 2.5). This is probably due to the fact that posterior legs are less perpendicular to the longitudinal axis of the body and therefore a larger proportion of the range of motion translates into lateral displacement instead of forward propulsion. From our observations, *L. emarginata* appear to make minimal use of their second and third legs during walking. The forward walking soldier crab *M. platycheles* was also reported to have larger ranges of motion for joints in the horizontal plane than for these joints in sideways walking species (Sleinis and Silvey, 1980).

### **Segment Lengths**

We found a positive correlation between the length of segments proximal to a joint and that joint's range of motion. This suggests that coupled with a larger range of motion is a longer

cavity housing the musculature responsible for the movement. This is similar to the increased length of the endophragmal compartments in *L. emarginata*.

### **Stance Width and Length**

Of the three species studied, *C. maenas* shows the most lateral placement of the dactyls (Fig 2.7Ai, D), whereas *L. emarginata* has the most symmetrical arrangement (Fig 2.7Aii, D, and E). This is not surprising since the legs of *L. emarginata* are arranged radially around the thorax. The distance between insertion points and the curvature of the ventral thorax provide *L. emarginata* with more room for proximal musculature than *C. maenas* (Fig 2.7Bi vs. ii). This parallels the observations on the endophragmal skeleton (Fig 2.3). In *C. maenas* and in *P. clarkii* the trend was less pronounced until the fifth legs, which were placed backward as much as the second legs were forward (Fig 2.7C, E). The distance between contralateral legs was longer for *C. maenas* than for the other two species (Fig 2.7). This leg arrangement is perhaps a reflection of the walking preference of *C. maenas* since it increases its ability to counteract drag forces in the sideways direction. A wider stance in the direction of drag forces implies that the center of mass would rest further inside the polygon of support and therefore be more stable (Alexander, 2002).

### **Contribution of Joints to Stance**

Our goal was to find whether there are morphological and functional differences between a forward walking brachyuran crab (*L. emarginata*), a forward walking macruran (*P. clarkii*), and a more typical brachyuran that walks sideways (*C. maenas*). We found some similarities between the two brachyurans, but also other similarities were shared between the forward-walkers. Individual joint angles at rest in the two crab species were similar. All the vertically moving joints were partially flexed, which resulted in each leg adopting an arc-shape. Joints bending in the horizontal plane were close to rest ( $180^\circ$ ), but appeared to contribute to an

increased sprawl of the dactyls and a larger stance base. Leg use differed in *P. clarkii* in that one joint (MC) was flexed more than any of the other vertically moving joints, giving the crayfish legs a L-shaped appearance. The ensuing reduction in stance width reduces *P. clarkii*'s stability in the sideway direction, but increases stability in the anterior-posterior direction by reducing drag. This occurs by having the legs closer to the thorax and by having the legs in line, both of which reduce the animal's frontal area.

The main differences in leg arrangements during stance between crab species lie in the two posterior limbs. Here, fairly similar dactyl placements were achieved by different means. In *C. maenas*, all the distal joints of legs four and five maximize the breadth of the stance. This distal displacement is necessary for stability when we examine how the legs insert in the thorax. The insertion points in *C. maenas* are more parallel to the longitudinal axis than in the other two species. This reduces the potential horizontal swing by the proximal (TC) joint. In *L. emarginata*, however, the legs exit the thorax more radially providing more separation between adjacent limbs, which indicates that the proximal joints and segments are already displaced caudally in posterior legs.

#### ***Libinia emarginata* and *Mictyris platycheles***

The skeletal similarities found between *L. emarginata* and *P. clarkii* are also present in another forward walking crab, *Mictyris platycheles* (Sleisin and Silvey, 1980). Both *L. emarginata* and *M. platycheles* crabs have orbicular thorax shape and legs arranged radially around the carapace instead of the parallel arrangement found in most sideway walking crabs. *M. platycheles* also has a larger range of motion than sideway walking crabs for joints propelling the animal forward. These similarities are more striking when one considers that *L. emarginata* and *M. platycheles* are not closely related species. Leg arrangement, thoracic elongation in preferred walking direction, and increased segmental ranges of motion appear to show adaptations in



forward walking crabs and macrurans. This suggests that these are important skeletal features subject to selective pressures.

### **Concluding Remarks**

Much of the work performed in crustacean locomotion took place under the assumption that all legs functioned in a similar fashion. Anatomical and physiological works often neglect to report which leg was chosen to study and the reason for that choice. The limbs of decapod crustaceans are subject to the same selective pressures that over time have produced the remarkable variety of arthropod forms we see in the world today.

Past assumptions often made on the structural and functional similarities between different legs need to be replaced by more detailed descriptions of each limb and its roles in stance and locomotion. Only by these means will we have the correct context in which to place the ever-increasing wealth of knowledge obtained from these organisms. Comparison between *L. emarginata* and closely related sideways walking crabs might prove useful in understanding the anatomical differences that were necessary for forward walking homoloid ancestors to become sideways walking brachyuran crabs.

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## **CHAPTER 3**

### **MUSCULAR ANATOMY OF THE LEGS OF THE FORWARD WALKING CRAB, *LIBINIA EMARGINATA* (DECAPODA, BRACHYURA, MAJOIDEA)**

## INTRODUCTION

Decapod crustaceans have been used successfully in the study of behavior and neuroscience for many decades (Atwood, 1977; Clarac et al., 1987; Hoyle, 1975; Huxley, 1880; Macmillan, 1975; Paul, 2006, Rathmayer and Bévengut, 1986). They perform interesting and diverse behaviors (Faulkes and Paul, 1997, 1998; Hoyle and Burrows, 1973; Hoyle 1973) with relatively simple and accessible nervous systems. Because of the diversity of research approaches that these organisms accommodate, they have contributed much to neuroethology (Atwood, 1977; Clarac, 1977; Macmillan, 1975; Sattelle and Buckingham, 2006). Despite the important progress achieved in diverse fields dealing with the study of animal behavior, our understanding of the muscular anatomy of these organisms has not kept up with advances in other aspects of neuroethology (examples of work in the field are Antonsen and Paul, 2000; Bévengut et al., 1983, Boxshall, 2003; Cochran, 1935; Hessler, 1982; Huxley, 1880; Pilgrim, 1964; Pilgrim and Wiersma, 1963), and remains patchy at best.

Research on the walking machinery of crustaceans is usually carried out on individual legs chosen for their accessibility and often under the assumption that different legs behave similarly. This assumption was supported in sideways walking brachyurans by the finding that different legs are used in similar fashion (with the exception of the specialized terminal pereopods) (Barnes, 1975; Burrows and Hoyle, 1973; Clarac et al., 1987). Sideway walking is bidirectional in nature (the same leg can be leading or trailing on different occasions), however, the same adaptations that allow animals to walk in opposite directions might also prevent the specialization of limbs for walking in a particular direction, as is often the case for animals that walk forward (Ritzmann et al., 2004). For example, animals that walk unidirectionally have limbs anterior to the center of mass that always pull, and limbs behind it that always push. This division in labor can lead to the specialization into hind and fore limbs observed in diverse taxa.

In crustaceans, forward walking crayfish (Jamon and Clarac, 1994, 1997) and lobsters (Macmillan, 1975; Ritzmann et al., 2004) have different legs that assume different roles in forward walking behavior.

Although capable of walking sideways, the portly spider crab (*L. emarginata*) is a brachyuran that walks preferentially forward (Schreiner, 2004; Vidal-Gadea et al., 2008). Spider crabs are majoids and as such are thought (Rice, 1983; Morrison et al. 2002) to be basal to the brachyurans. Because they are not laterally compressed (as is the case for many decapods), the thoracic nervous system of spider crabs is ventrally accessible without disruption of the walking musculature. The behavioral repertoire, accessible nervous system, and phylogenetic position of *L. emarginata* make it useful in the study of the neural control of legged locomotion. The present study is the second of a series looking at the anatomy and physiology of *L. emarginata*, and how they relate to its locomotor behavior. We have previously shown (Vidal-Gadea et al, 2008) that *L. emarginata* has skeletal adaptations that reflect its walking preference. We hypothesized that the forward walking spider crabs possess differences in musculature between anterior and posterior pereopods that reflect their specialization into fore limbs and hind limbs. As part of our ongoing neuroethological studies of walking behavior, we describe the complete muscular anatomy of the legs of *L. emarginata* in order to gain understanding of the adaptations that are concomitant with forward walking.

## **MATERIAL AND METHODS**

### **Animals Used**

*L. emarginata* (n=10) were obtained from the Marine Resources Center of the Marine Biological Laboratories in Woods Hole, Massachusetts, and kept at 20°C in artificial seawater.

## **Muscular Anatomy**

We used male and female crabs that ranged 7 to 10 cm in carapace length. Animals were euthanized by cooling and dissected dorsally. The carapace was removed and the animals were immersed in crab saline. Methylene blue enhanced contrast between the muscles. Photographs were obtained by mounting a digital camera on a dissecting microscope and used to generate musculature drawings in Corel Photo-Paint

## **RESULTS**

### **Pereopod Architecture**

As with other brachyurans, the five pereopods of spider crabs consists of six segments (Fig 3.1) that articulate with each other via bicondylar joints. This restricts the range of motion of each segment to a single plane. By having these planes positioned so that they are perpendicular to that of their neighbor, crustaceans manage to articulate their legs in any direction.

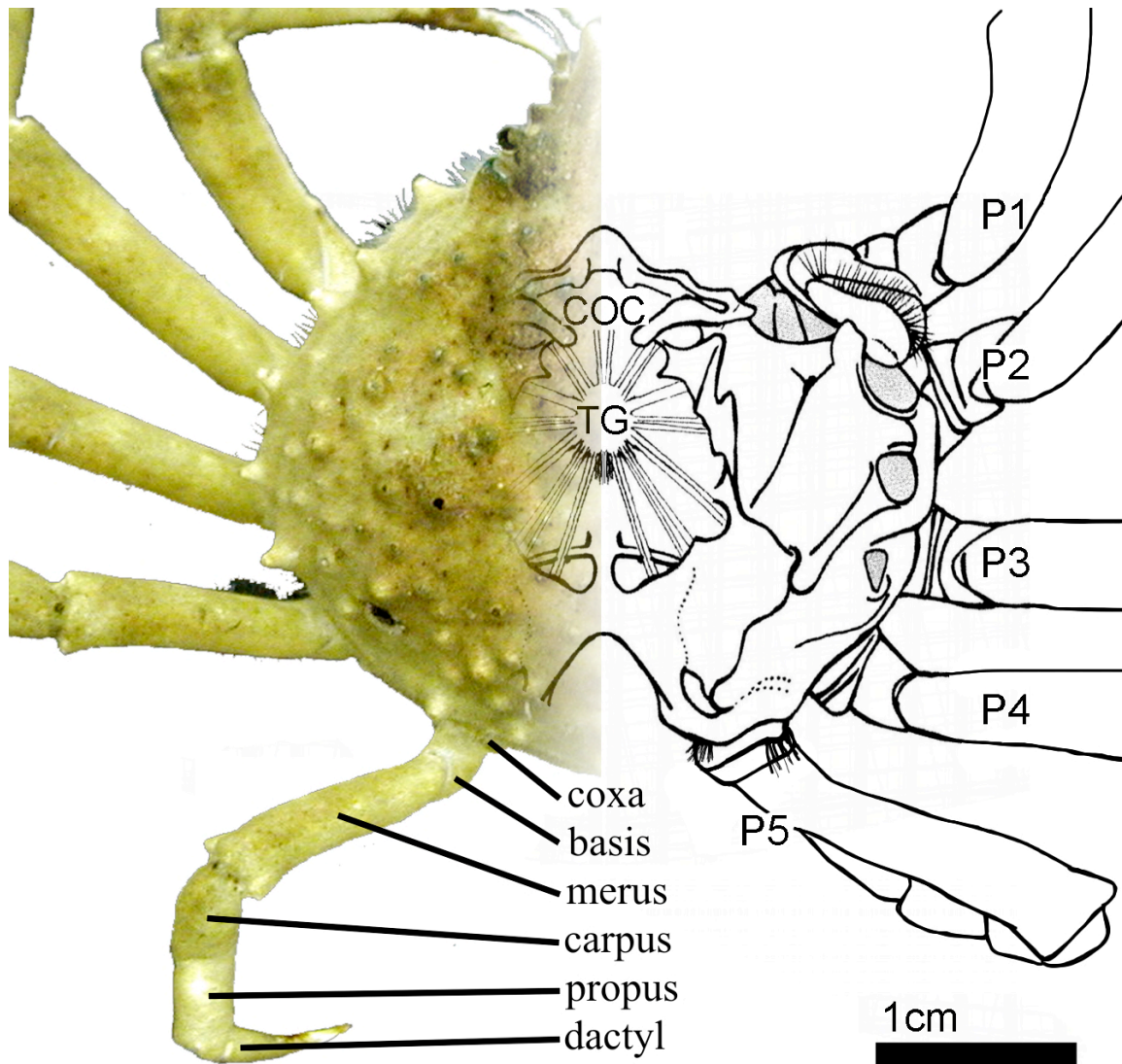
### **Distal Musculature**

The distal musculature of decapod crustaceans is highly conserved and was described in a number of diverse taxa (Atwood, 1977; Clarac and Vedel, 1971; Hessler, 1982; Wiersma and Ripley, 1952). With but a couple of exceptions, the distal musculature of spider crabs resembles that described for related species. All but two distal muscles have common features between the different pereopods. For this reason, the description of the distal musculature (unless stated) applies to all the pereopods of this animal.

### **Opener Muscle**

The opener muscle (Fig 3.2), responsible for abduction of the dactyl, originates on the dorsal half of the propodite segment and inserts on a single apodeme that is connected to the





**Figure 3.1.** Dorsal view of *Libinia emarginata* showing the underlying endophragmal skeleton and the leg segments, the walking legs (1-5), as well as the thoracic ganglia (TG), and circumesophageal connective (COC).

dorsal projection of the dactyl. In the first pereopod, this muscle is much reduced to accommodate the larger closer muscle.

### **Closer Muscle**

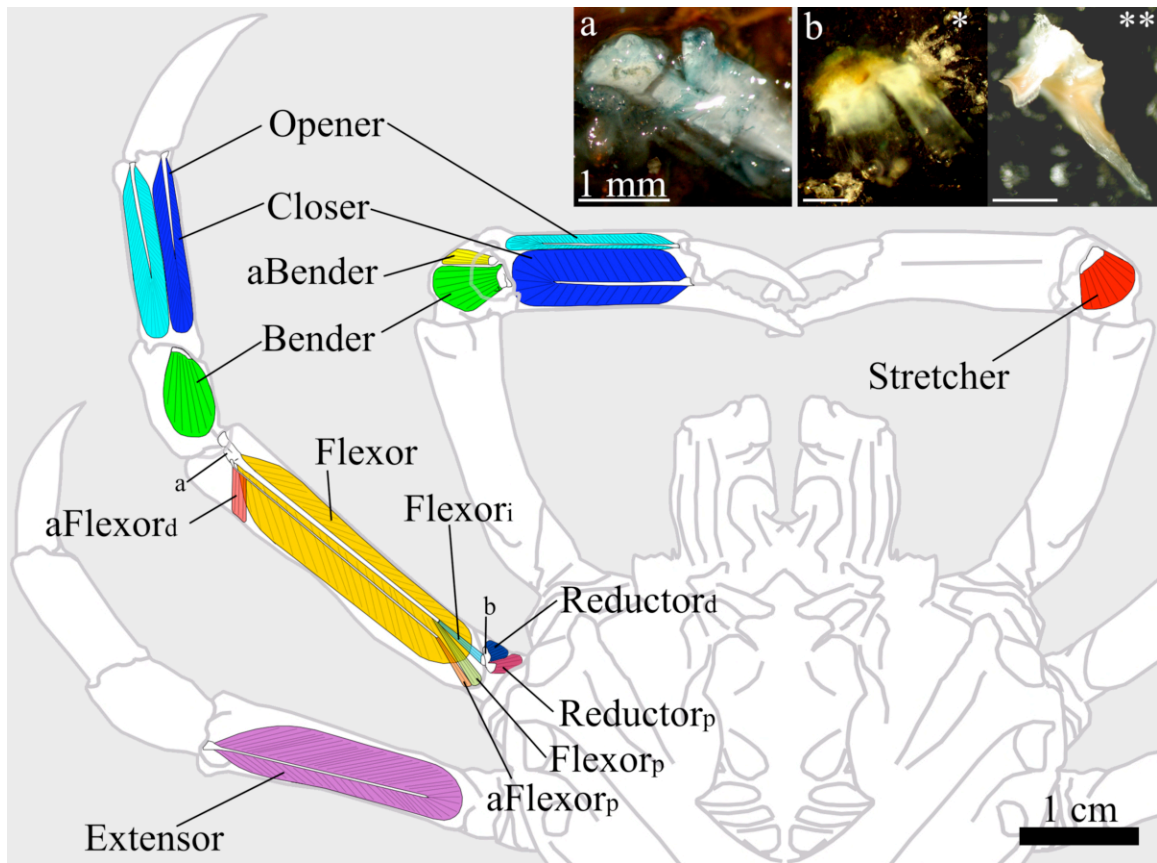
The closer muscle, which closes (adducts) the dactyl, originates on the ventral half of the propodite and inserts onto a single apodeme attached to the ventral projection of the dactyl. The enlarged first propodite of *L. emarginata* possesses a larger closer muscle that occupies much of the volume available in this segment. This particular muscle is further differentiated from other closers in that the muscle fibers are more oblique (and correspondingly shorter) than in other legs. The fiber arrangements in the first pereopod allow this muscle to accommodate a larger number of (shorter) fibers than homologs in posterior legs (Fig 3.2).

### **Stretcher Muscle**

The stretcher muscle is similar in each of the pereopods. It originates on the postero-proximal half of the carpopodite and inserts onto a single, wide apodeme connected by arthrodial membrane to an invagination on the posterior edge of the propodite (Fig 3.2). This muscle is responsible for adduction of the propodite.

### **Bender Muscles**

The bender muscles, responsible for flexing the propodite in the anterior direction and originate on the proximal and anterior edge of the carpopodite, insert onto a broad apodeme connected by arthrodial membrane to an invagination on the anterior edge of the propodite (Fig 3.2). Although this muscle is practically identical in pereopods 2 through 5, it is different in pereopod 1. Pereopod 1 has two bender muscles with distinct origins and apodemes (Fig 3.2). The largest bender in leg 1 is similar to the bender in the rest of the legs in origin, attachment and size. There is a smaller bender muscle that inserts on a smaller apodeme just dorsal to the



**Figure 3.2.** Dorsal view of the distal musculature of the legs of *Libinia emarginata*. The musculature is highly conserved and only shown for different legs when the trend varies (for the benders and closers of leg one). The insets show details on the proximal flexor apodeme (**a**), and the reductors apodemes (**b**) in dorsal (\*) and lateral (\*\*) views (see Table 3.1 for abbreviation key).

main bender muscle (Fig 3.2). I found these pair of muscles to also be present in the first pereopods of the green shore crab *Carcinus maenas*, and the crayfish *Procambarus clarkii* (data not shown).

### **Extensor Muscle**

The extensor muscle shares many features with the opener. It consists of a single muscle of dorsal (and proximal) origin in the meropodite segment, which inserts onto a single apodeme that is connected to the dorsal edge of the carpopodite by arthrodial membrane (Fig 3.2). The fibers of this muscle are long and run less perpendicular to the axis of the apodeme than flexor fibers.

### **Flexor Muscles**

The flexor muscle is anatomically the most complex of the distal muscles. It consists of two apodemes and five muscle heads.

#### Main Flexors

The main flexor muscle, together with the closer in leg 1, is one of the two largest distal muscles. It originates on the ventral half of the meropodite segment and inserts onto a long apodeme. The anterior half of this apodeme connects, via the arthrodial membrane, directly to the carpopodite. The posterior half of the main flexor apodeme inserts via a flexible ligament onto an anterior lip of the apodeme of the accessory flexor apodeme (see insert “a” in Fig 3.2). The flexor of *L. emarginata* differs from related species (Parsons and Mosse, 1982) in having two additional muscle bundles that insert onto the narrow proximal lip of the main flexor apodeme. A small muscle bundle originating proximally on the postero-ventral edge of meropodite (Flexor<sub>p</sub>, see Table 3.1 for abbreviations used) inserts on the posterior half of this lip. The third muscle bundle to insert on this apodeme is a small group of intersegmental fibers

(Flexor<sub>i</sub>) that arise on a projection of the reductor muscle apodeme and insert on the anterior edge of the flexor apodemal lip (see insert “b” in Fig 3.2).

### Accessory Flexors

Govind and Wiens (1985) previously looked at the innervation of the accessory flexors in *L. emarginata* and found it to be similar to that of related species (Barth, 1934; Melvin and Cohen, 1963; Clarac and Vedel, 1971;) The accessory flexor muscle consists of two muscle bundles inserting onto a long, thin apodeme. This apodeme (see insert “a” in Fig 3.2) widens anteriorly before connecting to the carpopodite to connect (via a flexible ligament) to the apodeme of the main flexor muscle. The most proximal of these bundles (aFlexor<sub>p</sub>) originates just posterior to Flexor<sub>p</sub> and, similarly to them, inserts onto the proximal end of the accessory flexor apodeme (Fig 3.2). The most distal of the accessory flexor muscle heads (aFlexor<sub>d</sub>) is a short, laminar bundle of fibers that originates on the posterior and distal side of the meropodite.

### **Reductor Muscles**

The reductor muscles are responsible for rotating the meropodite anteriorly (thus pitching the axis of the leg forward). These muscles consist of two distinct muscle heads that originate proximally on the dorsal anterior end of the basi-ischiopodite side by side and run in parallel to their insertion on two, partially fused apodemes that attach to the dorsoposterior edge of the meropodite (see insert “b” in Figure 3.2). From these apodemes, a perpendicular projection arises proximally where the fibers of the Flexor<sub>i</sub> bundle attach.

### Proximal Musculature

#### **Notes on the Skeletal Structure**

Most of the proximal musculature in decapod limbs arises from skeletal structures in the endophragmal skeleton. Before describing this musculature, we give a brief overview of the

**Table 3.1.** Abbreviations used in this study.

<b>aBender</b>	Accessory Bender muscle (Leg 1)
<b>aFlexord</b>	Accessory Flexor muscle, distal bundle
<b>aFlexorp</b>	Accessory Flexor muscle, proximal bundle
<b>BI-M</b>	Basischium-merus joint
<b>CB</b>	Coxa-basis joint
<b>CP</b>	Carpus-propus joint
<b>Flexori</b>	Main flexor muscle, intersegmental bundle
<b>Flexorp</b>	Main flexor muscle, proximal bundle
<b>MC</b>	Merus-carpus joint
<b>PD</b>	Propus-dactyl joint
<b>P1-5</b>	Pereopods one through five
<b>Reductord</b>	Distal reductor muscle
<b>Reductorp</b>	Proximal reductor muscle
<b>TC</b>	Thoracico-coxal joint
<b>TG</b>	Thoracic ganglia

endophragmal skeleton from which of it arises. A more thorough description may be found in Vidal-Gadea et al., 2008. We employ the nomenclature used by Huxley (1880) and Cochran (1935). Briefly, the proximal musculature of each leg originates within the coxopodite or the thorax. In the thorax, each segment (somite) is flanked by endophragmal skeleton. The bottom of each somite is termed the sternite, and the top of each endophragmal somite is called the epimeral plate. Vertical ribs arise from the sternite between each somite. These are called endosomites, and can be anterior or posterior within a somite. Approximately half way between the sternite and the epimeral plate, horizontal projections of skeleton invaginate from the endosternites; these are called mesophragm (if medial) or paraphragm (if distal). These projections divide the endophragm into a ventral and a dorsal compartment. The epimeral plate

is connected to the ventral endophragm by its own vertical ribs, called endopleurites, which constitutes the basic structure of the endophragmal skeleton. The dorsal endophragm of anterior segments, however, can overlap the ventral endophragm of posterior segments. Whenever necessary, we clarify differences in endophragmal structure between the pereopods where the musculature attaches.

### **Nomenclature Used**

Unlike the distal musculature, the proximal musculature of the legs of spider crabs show important differences between the different pereopods. Because of the number and complexity of the proximal musculature, we will describe each group of muscles for each leg. It is important to note that the legs of spider crabs are more radially arranged around the thorax than those of sideways walking crabs (Vidal-Gadea et al., 2008). This indicates that for muscles moving the legs in the forward and backward direction (promotors and remotors), the orientation of the force vectors created by each muscle bundle must be considered in combination with the insertion angle of the limb. We employ a muscular nomenclature based on the anatomical properties of the muscles, without implying homology of function or development. We distinguish between muscle bundles that arise and/or insert on distinct skeletal structures. We use the nomenclature employed by Antonsen and Paul (2000), but have expanded it into a naming system that simplifies the cumbersome task of naming different muscle bundles (Table 3.2). This system is as follows. Each muscle bundle is given a series of letters that describe its function, position, origin, and the location of its base, which unambiguously identifies the anatomical characteristics of the bundle. The first letter is capitalized and describes the muscle group to which the bundle belongs. If more than one muscle is present (one muscle is defined here as all muscle bundles sharing a common apodeme), a subscript letter will indicate the relative placement compared with the other muscles in the group. If there is only

one muscle, this subscripted letter will be absent. The third and fourth letters indicate the location within the skeleton from where muscle bundles arise. The fifth and last letter (if present) will be in subscript to indicate the relation of the origin of the bundle to other bundles of the muscle on the same structure. As an example of this naming system, consider **D<sub>m</sub>cx<sub>v</sub>**. The capitalized letter D indicates that this is a bundle of the depressor muscle. The subscripted letter **m** indicates this as a medial (as opposed to anterior or posterior) bundle. The third and fourth letters “**cx**” indicate that this bundle originates within the coxa, and the subscripted letter “**v**” indicates that this bundle has a ventral origin.

**Table 3.2.** Naming abbreviations used for proximal musculature.

1st	definition	2nd	definition	3rd	definition	4th	definition
<b>D</b>	Depressor	<b>a</b>	anterior	<b>es</b>	endosternite	<b>a</b>	anterior
<b>L</b>	Levator	<b>m</b>	medial	<b>cx</b>	coxa	<b>p</b>	posterior
<b>R</b>	Promotor	<b>p</b>	posterior	<b>st</b>	sternite	<b>v</b>	ventral
<b>P</b>	Remotor			<b>ep</b>	epimeral plate	<b>d</b>	dorsal
				<b>pp</b>	paraphragm	<b>m</b>	medial
				<b>mp</b>	mesophragm	<b>r</b>	distal
				<b>fr</b>	foramen		
				<b>el</b>	endopleurite		

Naming key used for the bundles of the proximal musculature of *L. emarginata*. The first (capitalized) letter describes the group to which the bundle belongs. The second (subscripted) letter signals the presence and relative location of multiple apodemes for a muscle. The third and fourth letters indicate the skeletal structure from where the bundle originates. The final (subscripted) letter indicates the relative position of the bundle origin.

### **The First Pereopod**

The first pereopods are specialized into chelipeds, but are also used in walking (Chapter 5). They have several anatomical differences from the posterior pereopods.



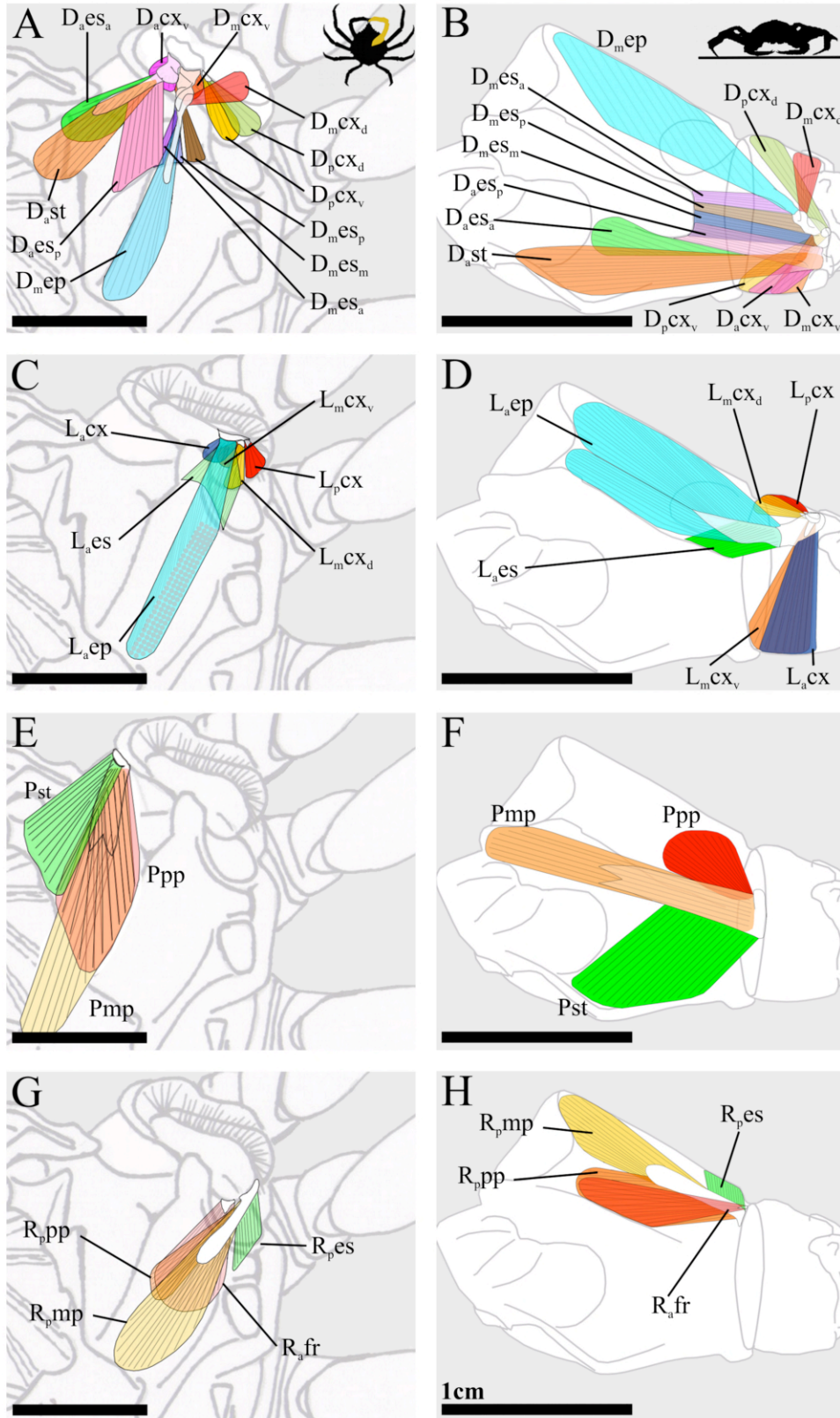
## Depressors

The depressor musculature of the first pereopod is the most complex of the muscles that move the legs of *L. emarginata*. Three distinct apodemes converge on the ventral lip of the basopodite (Fig 3.3A and B). Because there is a posterior rotation of the axis of the leg, the depressor site of apodeme insertion lies anterior (as well as ventral) within the coxa. Of the twelve muscle heads described for the depressor musculature of this pereopod, only one ( $D_{mep}$ ) is housed on the dorsal endosomite, with the rest of the musculature being housed either in the coxa or on the ventral endophragmal cavity.

### Anterior Depressors

The most anterior apodeme serves as attachment site for four muscle bundles, three originating in the thorax and one contained within the coxa. The coxal head of the anterior depressor apodeme ( $D_{acx_v}$ , see Table 3.2) originates immediately below the insertion point of its apodeme on the ventral surface of the coxopodite. It extends dorsally a short distance to insert onto the flattened ventral surface of the apodeme. The remainder of the muscle heads of the anterior apodeme originate within the thorax. The most anterior of these is  $D_{aes_a}$ , which originates distally from the anterior endosternite and separates the first pereopod from the third maxilliped. Thinner and longer fibers comprise the next muscle head attached to the anterior apodeme, the  $D_{ast}$ . These fibers originate from the most ventral and proximal surface on the anterior endophragmal compartment, the sternite. The fourth muscle head inserting onto the anterior depressor apodeme is  $D_{aesp}$ , which consists of a bundle of horizontally and anteriorly inserting fibers originating from the distal part of the medial endosternite and inserting onto the posterior lip of the apodeme.

**Figure 3.3.** Proximal musculature of the first pereopod of *Libinia emarginata*. Dorsal (left column) and frontal (right column) views of the muscle bundles comprising the depressor (**A, B**); levators (**C, D**); promotors (**E, F**); and remotor (**G, H**) muscles. Refer to Table 3.2 for muscle bundles abbreviation key.



## Medial Depressors

The medial apodeme is moved by six different muscle heads; two of these reside entirely within the coxa and four arise in the thorax. The most ventral of the coxal heads is  $D_{mcx_v}$ , originating on the ventral surface of the coxa (posteriorly adjacent to  $D_{acx_v}$ ) and running dorsally to insert on the ventral surface of the apodeme as it inserts onto the lip of the basipodite. The second of the coxal-bound muscle heads is  $D_{mcx_d}$ . It originates posteriorly on the distal and dorsal side of the coxa and inserts anteriorly and ventrally to its insertion site on a dorsally oriented lip of the apodeme. The next three medial muscle heads have similar origins and insertions and are only described separately because of the complexity of the apodemal surfaces upon which they insert. The most anteriorly originating of these heads is  $D_{mes_a}$  which originates from the vertical distal surface of the endosternite before it turns from facing anteriorly to antero-medially. The  $D_{mes_a}$  inserts horizontally to its insertion site on the ventral edge of the apodeme as it connects through a flexible ligament to the section of apodeme that attaches via connectives to the ventral lip of the basipodite. The next muscle head lying in close proximity to  $D_{mes_a}$  is  $D_{mes_m}$ . It originates ventrally and distally to  $D_{mes_a}$  on the endosternite and inserts ventrally and anteriorly to it on the ventral surface of the apodeme that contacts the basis. Just before the medial apodeme contacts the basipodite, it sends a ventral lip to which the third muscle head,  $D_{mes_p}$ , attaches. This muscle head is the most posterior of the three, and like the previous two, inserts horizontally from its origin on the most distal portion of the endosternite (facing anteriorly) to its insertion site. The final and largest of the medial depressor muscle heads is  $D_{mep}$ . This muscle head originates on the ventral surface of the epimeral plate and inserts on the dorsal surface of the medial apodeme.

### Posterior Depressors

The third depressor muscle is fully contained within the coxa. Its apodeme is much smaller than the other depressor apodemes and it is the insertion site for two distinct muscle heads. The most anterior of these is  $D_{p}cx_v$  originating medially on the postero-ventral surface of the coxa.  $D_{p}cx_v$  travels in the anterior, medial and distal direction to insert on the anterior surface of its apodeme. The second muscle head ( $D_{p}cx_d$ ) inserts on the posterior edge of the apodeme and originates dorsally on the postero-distal side of the coxa.

### **Levators**

The levator musculature is simpler than that of the depressors but it also has three apodemes that lie adjacent to each other on the postero-dorsal edge of the coxa. Six muscle-heads insert onto these apodemes, of which two originate in the thorax (Fig 3.3C, D).

### Anterior Levators

The anterior levator muscle is composed of one large apodeme upon which three muscle heads attach. The largest bundle ( $L_{aep}$ ) originates dorsally along the ventral surface of the epimeral plate (posterior to  $D_{mep}$ ). From its origin  $L_{aep}$  inserts distally and ventrally on the dorsal surface of the apodeme. The second bundle attaching on this apodeme is  $L_{aes}$ , which originates on the distally facing surface of the endosternite and travels horizontally a short distance to its insertion site along the ventral and posterior aspects of the apodeme. The last and smallest of the anterior levator heads is the coxa-bound  $L_{acx}$ . This short bundle originates on the ventral surface of the coxa, directly beneath the apodeme, and travels dorsally to its insertion site on the ventral surface of the apodeme.

### Medial Levators

The medial apodeme is much smaller than its anterior counterpart and connects to a posterior projection from it via a flexible ligament. The apodeme has a small horizontal surface

from which two attachment lips project. The most anterior of these exits ventrally and is the site of attachment for the ventrally, while the posterior exits in a slightly dorsal direction. Two short but wide bundles attach to these lips. The most anterior of these bundles is the  $L_{mcx_v}$ , which originates ventrally on the proximal and medial surface of the coxa. The second bundle is the  $L_{mcx_d}$ , which originates dorsally on the proximal and posterior surface of the coxa and travels in the ventral, distal and anterior direction to its insertion site.

### Posterior Levator

The posterior levator apodeme is small and, like the medial apodeme, is connected to a posteriorly projecting lip from the anterior apodeme. This apodeme is oriented posteriorly and is the site of attachment for a single muscle head,  $L_{pcx}$ . This muscle originates on the dorsal and posterior surface of the coxa and inserts anteriorly and ventrally to its insertion site.

### **Promotors**

The promotor musculature is fully contained within the thorax (Fig 3.3E, F). Because the axis of the leg is rotated slightly backward, the promotor apodeme attaches not just anteriorly, but also dorsally. Flexion of the promotor musculature results in a combination of promotion and elevation of the limb. Three distinct muscle bundles attach to the promotor apodeme. The most anterior of the promotor heads ( $P_{st}$ ) arises from the anterior sternite, adjacent to the anterior somite. From its origin, this bundle travels dorsally and distally to its insertion site on the ventral surface of the apodeme. The longest of the promotor bundles is  $P_{mp}$ . This bundle originates medially and posteriorly on the mesophragm (which actually overlaps the two posterior somites). The  $P_{mp}$  is composed of long, thin muscle fibers that insert on the dorsal surface of the apodeme. The most posterior of the three promotor muscle heads is  $P_{pp}$ , and it originates along the vertical surface of the distal endopleurite and paraphragm.

## **Remotors**

The remotor musculature of the first pereopod is composed of two muscles with two apodemes (Fig 3.3G, H). The anterior remotor muscle head  $R_{afr}$  originates around the dorsal edge of a paraphragmal foramen, located distal and posterior in the somite. This muscle inserts horizontally on the anterior apodeme. The three remaining muscle bundles attach to the most posterior (and larger) apodeme. The largest of these three bundles is  $R_{pmp}$ , which originates dorsally, medially, and posteriorly in the somite, from the mesophragmal surface to its insertion on the dorsal aspect of the apodeme. A second ( $R_{ppp}$ ) bundle originates more distally on the paraphragm (anterior to  $R_{afr}$ ) and inserts on the anterior lip of the apodeme. The last (and smallest) of the remotor muscles ( $R_{pes}$ ) originates distally on the endosternite and travels to its insertion on the posterior edge of the apodeme (just before it connects to the coxa).

## **The Second Pereopod**

The second pereopods are the first pair of legs specialized for walking (unlike the chelipeds) and as such share many features with the musculature of the remainder of the pereopods. Like the first pereopods, however, the second pereopods exit the thorax facing forward (Fig 3.1, 3.4).

## **Depressors**

These pereopods, like all posterior legs, are distinct from the first in that they lack the medial depressor muscle. Only two apodemes serve as attachment sites for the depressor musculature, which are anatomical homologs of the anterior and posterior depressors in leg one.

## **Anterior Depressors**

The most anterior apodeme is proximally articulated and serves as the attachment site for six muscle bundles. Three of these originate entirely within the thorax, and the remaining three are wholly housed in the coxa. The largest of these bundles is the  $D_{ast_m}$ , which originates on the

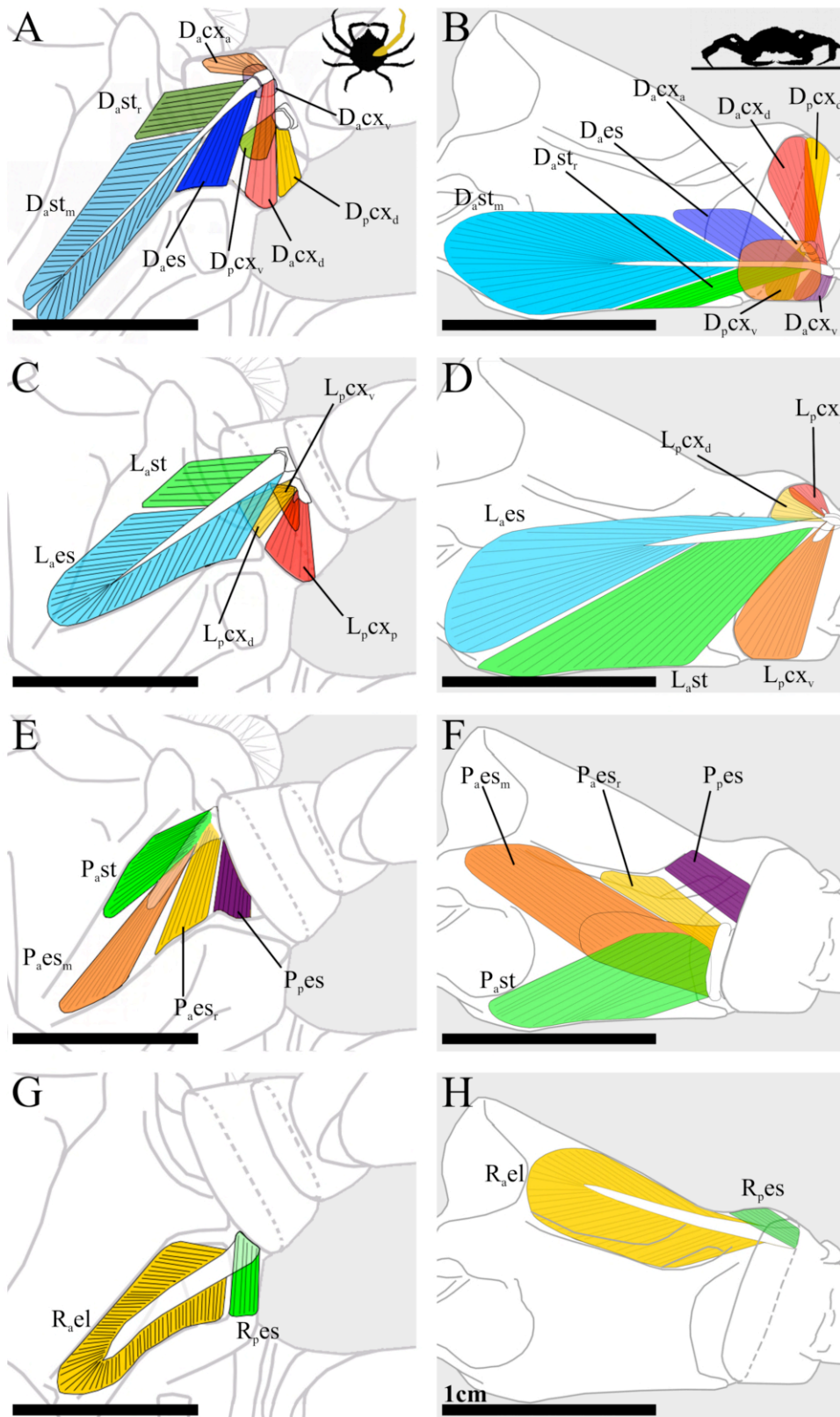
anterior and posterior endosternites (adjacent to leg 1) as well as on the ventral sternite (Fig 3.4A, B). The  $D_{ast_m}$  bundle attaches to the apodeme after it exits the coxal cavity. A second smaller bundle originates distally on the posterior endosternite after it turns to face anteriorly; this is  $D_{aes}$ . This second bundle originates from the same region where the  $D_{mes}$  bundles originate in leg 1, and inserts onto the posterior edge of the proximal apodeme which is still within the coxa. The third and last of the depressor bundles originating in the coxa is  $D_{astr}$ . This short bundle originates on the distal edge of the anterior endosternite and inserts onto the anterior edge of the apodeme still within the coxa. The anterior apodeme is connected via a flexible ligament to a smaller calcified section of apodeme that makes contact with the basis, and which is site of attachment for the coxa-bound anterior depressor bundles (Fig 3.4A). The most anterior of these bundles is  $D_{acxa}$ , which originates on the anterior side of the coxa and inserts posteriorly to its insertion on the anterior edge of the apodeme. The most ventral of the bundles,  $D_{acxv}$ , originates ventral to the apodeme and inserts on its ventral surface. The last and longest of the coxa-bound depressor bundles is  $D_{acxd}$ , which originates medially on the posterior and dorsal surface of the coxa.

#### Posterior Depressors

The posterior depressor apodeme is articulated and is not in close proximity to the anterior apodeme. Two muscle bundles attach to this apodeme. The most anterior bundle is the  $D_{pcx_v}$ , which originates proximally on the ventral lip of the coxa and travels distally to its insertion on the anterior part of the apodeme. The second bundle of the posterior depressor, the  $D_{pcx_d}$ , originates on the posterior and dorsal face of the coxa from where it travels anteriorly and ventrally to its insertion site on the posterior half of the apodeme.



**Figure 3.4.** Proximal musculature of the second pereopod of *Libinia emarginata*. Dorsal (left column) and frontal (right column) views of the muscle bundles comprising the depressor (**A**, **B**); levators (**C**, **D**); promotors (**E**, **F**); and remotor (**G**, **H**) muscles. Refer to Table 3.2 for muscle bundles abbreviation key.



## **Levators**

There are two levator muscles in the second pair of pereopods, the anterior and the posterior levators. The anterior levator musculature originates in the thorax while the posterior levators are confined to the coxa. The posterior apodeme is interesting in its structure and looks like a fusion of the medial and posterior apodemes in leg one (Fig 3.4C, D).

### Anterior Levators

The anterior levator muscle is composed of one large apodeme upon which two muscle heads attach. The largest of these bundles is the  $L_{aes}$ , which originates from the anterior and posterior endosternites. This bundle inserts onto the posterior side of the apodeme and onto the distal half of the anterior side of the apodeme. The second bundle attaching on this apodeme is  $L_{ast}$ , which originates distally on the anterior sternite and travels distally to its insertion site along proximal anterior aspect of the apodeme. The anterior apodeme is continuous via a flexible ligament with a smaller apodeme that connects with the basipodite.

### Posterior Levators

The posterior apodeme is loosely shaped like a letter “T” with one bundle inserting onto the left, and a second onto the right halves of the top. The last bundle inserts onto the bottom part (Fig 3.4C). The most anterior of these bundles ( $L_{pcxd}$ ) originates proximally on the dorsal lip of the coxa and inserts onto the anterior side of the apodeme. The posterior bundle ( $L_{pcxp}$ ) originates medially on the posterior and dorsal edge of the coxa and inserts on the posterior (and horizontal) lip of the apodeme. The ventral lip of the apodeme is site of attachment for the  $L_{pcxv}$  bundle, which originates ventrally on the proximal surface of the coxa.

## **Promotors**

The promotor musculature in the second pereopod is composed of two muscles and four thorax-bound muscle heads (Fig 3.4E, F). Contraction of the promotor musculature results in a

combination of promotion and elevation of the limb due to the posterior rotation of the axis defined by the condyles.

#### Anterior Promotor

The anterior promotor muscle has three distinct bundles. The most anterior of the promotor heads (Past) arises from the anterior surface of the ventral sternite, adjacent to the anterior somite. From its origin, this bundle inserts dorsally and distally on the ventral surface of the apodeme. The longest of the promotor bundles is P<sub>aes<sub>m</sub></sub>. This bundle originates medially and posteriorly on the anterior aspect of the posterior endosternite and is composed of long, thin muscle fibers that insert on the dorsal aspect of the apodeme. The last of the anterior promotor bundles is P<sub>aes<sub>r</sub></sub> which originates distally on the posterior endosternite before it turns from facing medially to facing anteriorly.

#### Posterior Promotor

The posterior promotor consists of a single muscle bundle that originates distally on the anterior facing surface of the posterior endosternite (distally adjacent to P<sub>aes<sub>r</sub></sub>). This bundle does not insert onto an apodeme, but directly onto the dorsal lip of the coxa (posterior to the insertion of the anterior apodeme).

#### **Remotors**

The remotor musculature lies dorsally and posterior within the somite. In the second pereopod there are two muscle bundles associated with the single remotor apodeme (Fig 3.4G, H). The largest of the remotor bundles is Rel, which originates on the posterior and medial endopleurites. The second remotor bundle (Res) originates from the distal-most surface of the posterior endosternite and travels horizontally to its insertion on the dorsal edge of the apodeme just before it connects with the coxa.

### **The Third Pereopod**

Although there are some important differences between the musculature of the second and third pereopods, the overall number and characteristics of the proximal musculature is similar between these two pereopods. The third leg is unlike the rest of the pereopods in that it exits the thorax perpendicular to the longitudinal axis of the animal.

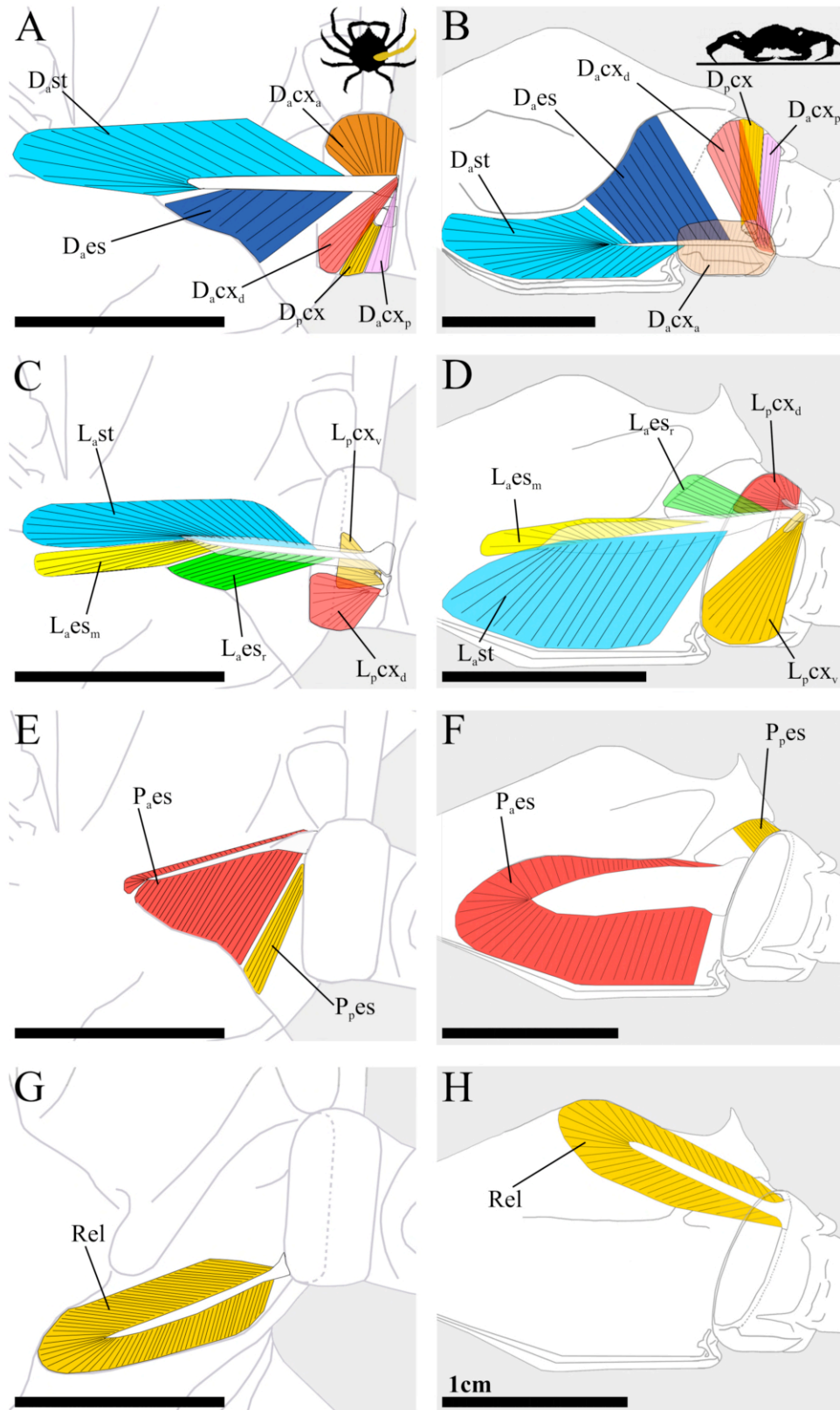
#### **Depressors**

As in the second pereopod, there are two depressor muscles in the third leg, the anterior depressor to which five muscle bundles attach, and the posterior depressor with its single bundle (Fig 3.5A,B).

#### **Anterior Depressor**

The anterior depressor in leg 3 has two thoracic bundles and three coxal bundles (Fig 3.5 A, B). The largest of the bundles is  $D_{ast}$  which is the anatomical homolog of  $D_{ast_m}$  in leg one. The  $D_{ast}$  bundle originates on the sternal and endosternal surfaces and inserts on the anterior face of the apodeme. The second (and last) bundle of the anterior depressor to originate in the thorax is  $D_{aes}$ . This bundle is anatomically homologous to  $D_{aes}$  in leg one. The largest of the coxa-bound bundles of the anterior depressor is  $D_{acx_a}$ , which is homologous to the one with the same name in leg 2. The second bundle in the coxa ( $D_{acx_d}$ ) is also present in leg 2 and has similar origin and attachments. The last bundle of the anterior depressor,  $D_{acx_p}$ , is not present in the second pereopod,  $D_{acx_p}$ . This bundle originates posteriorly on the dorsal and distal edges of the coxa and inserts just behind  $D_{acx_d}$  on the posterior lip of the apodeme as it connects the basis. In addition to not having the  $D_{acx_v}$  bundle, the apodeme of this muscle is not articulated as is the case in leg two.

**Figure 3.5.** Proximal musculature of the third pereopod of *Libinia emarginata*. Dorsal (left column) and frontal (right column) views of the muscle bundles comprising the depressor (**A, B**); levators (**C, D**); promotors (**E, F**); and remotor (**G, H**) muscles. Refer to Table 3.2 for muscle bundles abbreviation key.



### Posterior Depressor

A single muscle bundle is associated with the posterior depressor apodeme. This bundle ( $D_{pcx}$ ) originates from the posterior coxal side (dorsally) between  $D_{acx_d}$  and  $D_{acx_p}$  and inserts anteriorly and ventrally on a small, articulated apodeme (Fig 3.5A, B).

### **Levators**

Like the second leg, the third pereopod has two levator muscles. The anterior muscle has three bundles that are entirely thoracic, while the posterior muscle has two within the coxa (Fig 3.5C, D).

### Anterior Levator

The most anterior and largest of the bundles is  $L_{ast}$ . This bundle arises along the anterior edge of the sternite and inserts on the anterior aspect of the apodeme. The second largest of the levator bundles is  $L_{aes_m}$ , which originates medially on the posterior endosternite and inserts horizontally on the distal edge of the apodeme. The last of the thoracic levators is  $L_{aes_r}$ . It, like  $L_{aes_m}$ , also originates on the posterior endosternite, but more distally as the endosternite turns posteriorly.

### Posterior Levator

The apodeme of the posterior levator appears to be the result of the fusion between two smaller apodemes after the anterior component turned to point ventrally and the posterior to point dorsally. Two muscle bundles comprise the posterior levator. The most anterior of these is  $L_{pcx_v}$ , which originates ventrally on the proximal surface of the coxa and inserts on a ventral projection of the apodeme that lies anteriorly. The second posterior depressor bundle is  $L_{pcx_d}$ . This bundle arises proximally from the posterior and dorsal side of the coxa and inserts distally, ventrally, and anteriorly to its insertion site on a horizontal projection from the apodeme.



## **Promotors**

Two promotor muscles occur on the third pereopod, one anterior and one posterior (Fig 3.5E, F). The anterior promotor consists of a single apodeme and a continuous bundle of fibers  $P_{aes}$  that originate horizontally from the posterior endosternite and ventrally from the sternal surface. The much smaller posterior promotor,  $P_{pes}$ , originates on the posterior endosternite distally to  $P_{aes}$  and inserts directly onto the coxa just posterior to the site where the apodeme of the anterior promotor does.

## **Remotors**

The single remotor muscle in the third pereopod ( $Rel$ ) originates from the posterior and medial endopleurites (dorsally) as well as on the epimeral plate. This muscle inserts (via its apodeme) on the dorsal and posterior lip of the coxa (Fig 3.5G, H).

## **The Fourth Pereopod**

The fourth pereopod follows the radial arrangement of the legs around the thorax and is nearly perpendicular to the axis of leg two (Figs 3.4, 6).

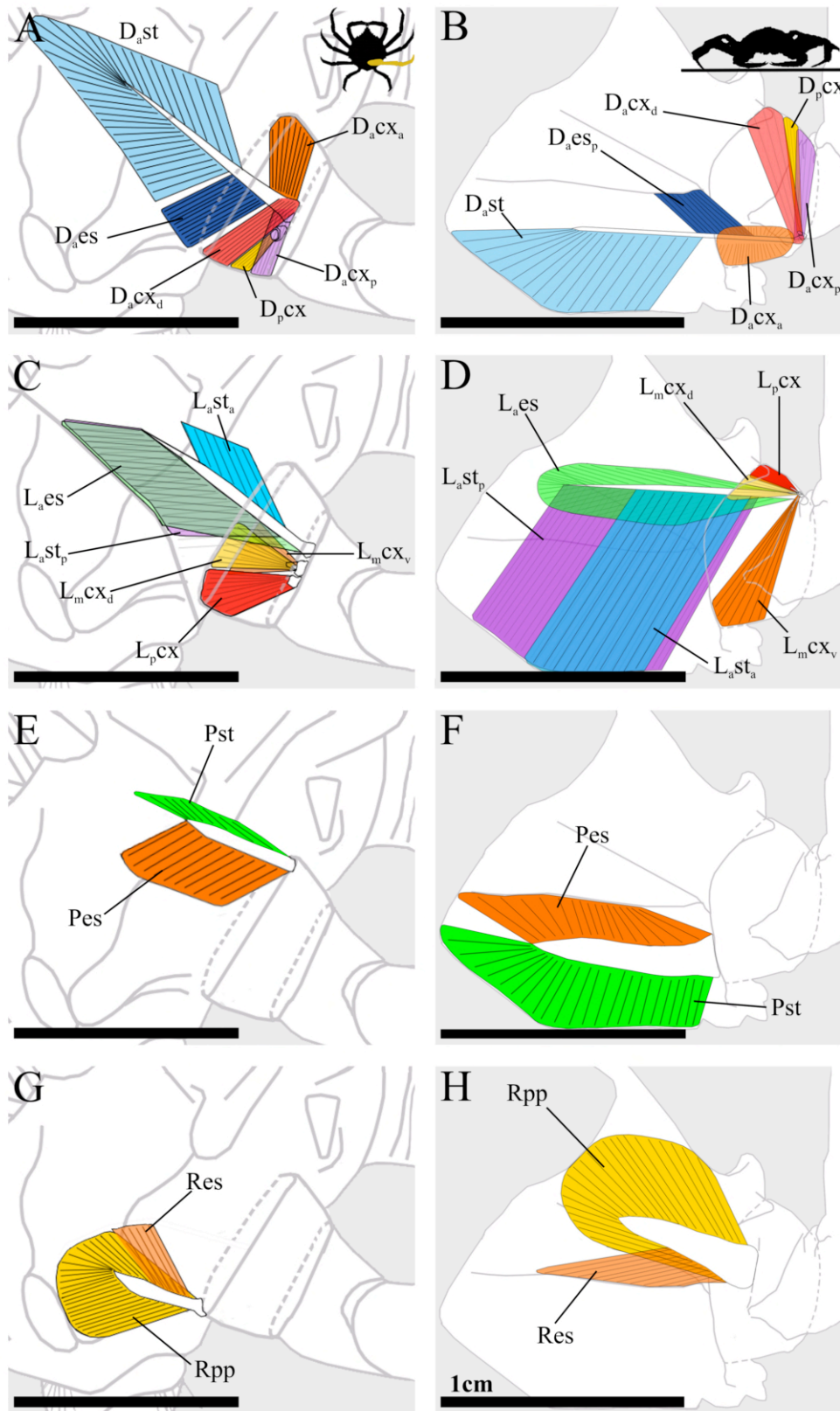
## **Depressors**

The depressor musculature of the fourth pereopod is identical to that of the third pereopod in muscle bundle numbers, origins, and attachments (Fig 3.4A, 4B, 6A, and 6B). The main differences between these two pereopods is in relative size of the muscle bundles and the orientation assumed by the skeletal structures from which they originate (Fig 3.6A, B).

## **Levators**

The levator musculature of the third pereopod is composed of three apodemes, and six muscle bundles (Fig 3.6C, D).

**Figure 3.6.** Proximal musculature of the fourth pereopod of *Libinia emarginata*. Dorsal (left column) and frontal (right column) views of the muscle bundles comprising the depressor (**A**, **B**); levators (**C**, **D**); promotors (**E**, **F**); and remotor (**G**, **H**) muscles. Refer to Table 3.2 for muscle bundles abbreviation key.



### Anterior Levators

The anterior levator musculature is contained within the thorax. The most anterior of the three bundles is  $L_{ast_a}$ , which originates anteriorly on the distal surface of the sternite and inserts on the anterior edge of the apodeme. A second, horizontal bundle ( $L_{aes}$ ) originates on the posterior endosternite and inserts on the posterior aspect of the apodeme. The last of the anterior levator bundles is  $L_{ast_p}$ , which originates ventrally on the posterior surface of the sternite and inserts on the ventral side of the apodeme.

### Medial Levators

The medial levator musculature, contained within the coxa, is composed of a small apodeme inserting just posterior to the anterior apodeme and the two muscle bundles that insert on it. A ventrally originating bundle ( $L_{mcx_v}$ ) inserts from its origin on the medial proximal lip of the coxa to its insertion on a ventral projection from the apodeme. The last of the medial bundles,  $L_{mcx_d}$ , originates dorsally on the proximal side of the coxa and inserts distally to insert on the apodeme.

### Posterior Levator

The single posterior levator muscle ( $L_{p_{cx}}$ ) originates dorsally (posterior to  $L_{mcx_d}$ ) and inserts in the distal-ventral-anterior direction to its insertion onto a small apodeme adjacent to the medial apodeme.

### **Promotors**

A single promotor muscle occurs on the fourth pereopod (Fig 3.6E, F) with two muscle bundles. The most anterior of the promotor bundles is  $P_{st}$ , which originates ventrally on the anterior surface of the sternite and inserts dorsally along the anterior face of the apodeme. The second promotor bundle ( $P_{es}$ ) originates posteriorly on the surface of the posterior endosternite and inserts horizontally on the posterior side of the apodeme.

## **Remotors**

A single remotor muscle with two muscle bundles is present in this pereopod (Fig 3.6H). I). The smallest and most anterior of these bundles is Res, which originates on the side of the anterior endosternite and inserts anteriorly on the dorsal and proximal side of the apodeme. The last and largest of the remotor bundles in this pereopod is Rel, which occupies much of the dorsal-posterior endophragmal compartment in the somite. The Rel originates on the surrounding endopleurites and epimeral plate and inserts on the anterior and posterior apodeme.

## **The Fifth Pereopod**

The last walking leg is the smallest of all the pereopods and exits the thorax pointing almost posteriorly (Fig 3.7).

## **Depressors**

The depressor musculature of the fifth pereopod is similar to that of its neighbor pereopod save for the following exceptions. The D<sub>ast</sub> bundle originates on the sternal surface and also on the anterior endosternite and consequently lacks the D<sub>aes</sub> bundle present in the third pereopod (Fig 3.7A, B).

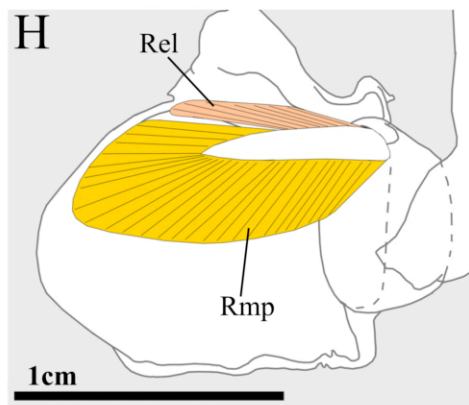
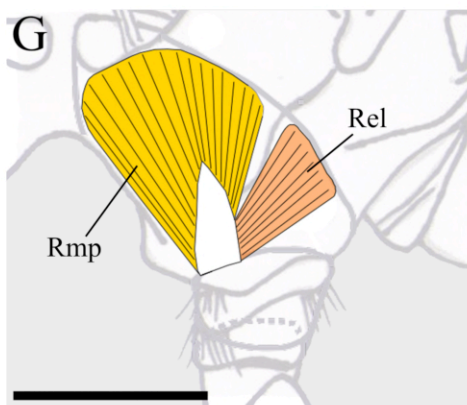
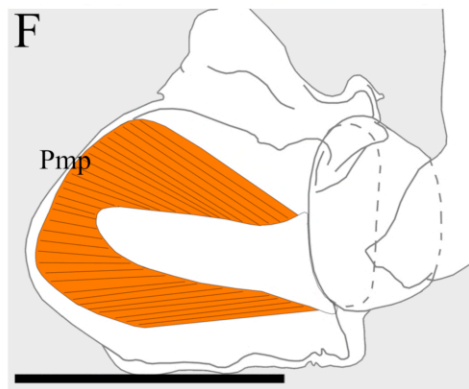
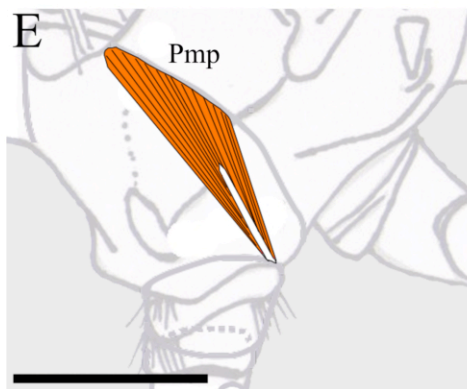
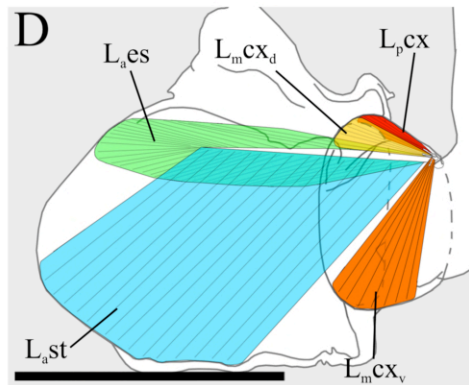
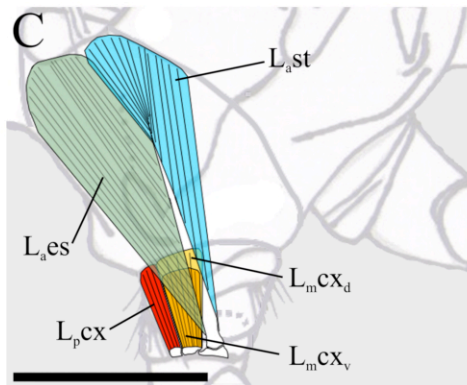
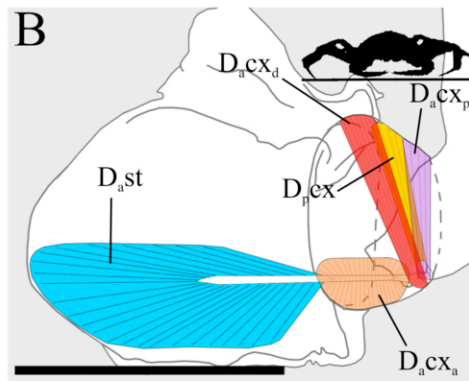
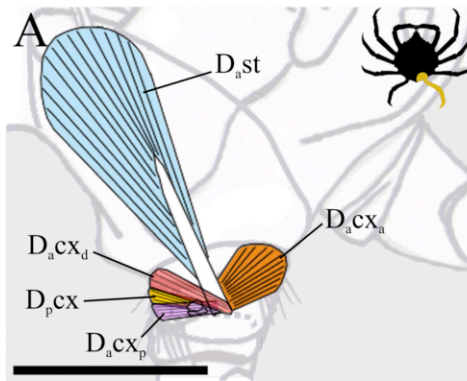
## **Levators**

Three apodemes occur for the levator musculature of the fifth pereopod to which five muscle bundles attach (Fig 3.7C, D).

## **Anterior Levators**

The anterior levator consists of two bundles that attach to a long apodeme. The most anterior (L<sub>ast</sub>) originates anteriorly on the ventral surface of the sternite and inserts on the anterior face of the apodeme. The second muscle bundle is L<sub>aes</sub>, which originates on the posterior endosternite and inserts horizontally on the posterior side of the apodeme.

**Figure 3.7.** Proximal musculature of the fifth pereopod of *Libinia emarginata*. Dorsal (left column) and frontal (right column) views of the muscle bundles comprising the depressor (**A, B**); levators (**C, D**); promotors (**E, F**); and remotor (**G, H**) muscles. Refer to Table 3.2 for muscle bundles abbreviation key.



### Medial Levators

Two medial levator bundles exist that share the same characteristics as those of the medial levators in the fourth pereopod.

### Posterior Levators

As with the medial levators, the posterior levator resembles that of the fourth pereopod except perhaps in having its apodeme placed more posteriorly.

### **Promotors**

A single, large promotor muscle arises from the anterior paraphragm and inserts on a large apodeme (Fig 3.7 E, F).

### **Remotors**

Two remotor muscle bundles occur on the fifth pereopod, which are anatomically similar to those in the fourth pereopod (Fig 3.7G, H). The most anterior of these two bundles, Rel, originates on the anterior endopleurite and attaches to the anterior face of the apodeme. The second and largest bundle, Rmp, originates on the mesophragmal surface and inserts on the posterior side of the apodeme as well as the distal half of the anterior side.

## **DISCUSSION**

### Distal Musculature

Although the distal musculature of *L. emarginata* is highly conserved between the pereopods, a few differences were observed between the first pereopod and the rest of the legs. One was the larger closer muscle in the first leg (Fig 3.2). This difference in size is expected from the specialization of the first pereopods into claws and is shared among many crustaceans (Boxshall, 2004). Perhaps the most interesting difference between the pereopods of *L. emarginata* is the second bender muscle in the first pereopod (Fig 3.2). Previous work on brachyuran distal leg musculature (Cochran, 1935) did not report this muscle. Furthermore,



physiological work (McDermott and Stephens, 1988) on the bender of *Pachygrapsus crassipes* also failed to report the second bender muscle. We found this muscle in the first pereopods of *P. clarkii* and *C. maenas* (not shown) as well as in *L. emarginata* (Fig 3.2). In his thorough work on eumalacostracan walking morphology, Hessler (1982) described musculature of the third pereopod of the *Palaemon squilla* (Eucarida) and noted the presence of an additional bender muscle in that species. Wiersma and Ripley (1952) described the presence of a second bender in all the members of the Natantia that they surveyed. From their description, and from the close resemblance that these muscles bear in the three species we examined, it seems likely that the two bender muscles are an ancestral trait and not later specializations.

Although the rest of the distal musculature was the same for all the legs, we found distal musculature differences between *L. emarginata* and other brachyurans described in the literature. The flexor musculature resembles more closely that of forward walking crustaceans than sideways walking brachyurans (Fig 3.2, Parsons, 1982; Parsons and Moose, 1982) in having multiple bundles, including an intersegmental one. Hessler (1982) described a flexor bundle in the primitive isopod *Janirallata* (Asellota) that extends through the ischium and inserts into the basis. This intersegmental bundle is also described for other decapod (*spelaegriphus lepidops*, Caridea) by Boxshall (2004) and is likely a retained ancestral trait. In *L. emarginata* this bundle inserts on a projection of the apodemes that hosts the two reductor muscles. It is noteworthy that these two muscle groups (reducers and flexors) receive innervation from a common thoracic nerve (Chapter 4).

### **Proximal Musculature**

The proximal musculature of crustaceans is much more complex than its distal counterpart. It is not surprising that previous work on the proximal musculature of crustaceans implicated it in the production of complex behaviors (Antonsen and Paul, 2000; Paul, 2006;

McVean, 1973; White and Spirito, 1973). We compared the number of muscle bundles in the proximal musculature of spider crabs to literature accounts from both closely related brachyurans that walk sideways and distantly related decapods that walk forward (Table 3.3). Forward walking decapods seem to have a larger number of proximal muscle bundles that insert onto discrete apodemes than their sideway walking counterparts.

### **Basal Muscles**

The basal musculature is responsible for raising the animal off of the substrate (depressors) and for lifting the leg during the swing phase during a step (levators). The proximal musculature differs considerably between the different pereopods (Figs 3.3-7). Because of its functional specialization, the first pereopod showed the deepest differences in number and morphology of proximal muscle heads (Table 3.4). Of all the legs, the depressor musculature of leg 1 had the most complex arrangement with 12 different bundles originating in the thorax and coxa (Fig 3.3A, B). The number of muscle heads described for the rest of the legs decreases in complexity and numbers posteriorly and from levator to promotor and remotor successively (in general). The large number of depressor muscle bundles is likely due to the function of these muscles in posture as well as locomotion. The levator musculature comprises one muscle arising in the thorax, and one or two arising in the coxa. Although most of the legs had two levator muscles as described for other decapod species in the literature (table 4) in spider crabs the posterior levator is further split into two separate muscles with different apodemes in the first and last pereopods. This second coxal levator muscle was reported for the green shore crab (Bévengut et al, 1983), although in *C. maenas* the additional bundle seems to be associated with the anterior levator muscle. The levator musculature of brachyurans was shown to be instrumental in the production of limb autotomy (McVean, 1973; McVean and Findlay, 1976;

**Table 3.3.** Comparison of proximal musculature across decapod species.

<i>Species</i>	<i>Carcinus</i>	<i>Callinectes</i>	<i>Libinia</i>	<i>Munida</i>	<i>Procambarus</i>	<i>Homarus</i>	<i>Palaemon</i>	<i>Janirallata</i>
	<i>maenas</i>	<i>sapidus</i>	<i>emarginata</i>	<i>quadrispina</i>	<i>clarkii</i>	<i>americanus</i>	<i>squilla</i>	<i>occidentalis</i>
Classification	Brachyura	Brachyura	Brachyura	Anomura	Astacidea	Astacidea	Caridea	Isopoda
	Eubrachyura	Eubrachyura	Eubrachyura	Galathoidea	Cambaridae	Nephropoidea	Palaemonoidea	Asellota
	Portunoidea	Portunoidea	Majoidea	Galatheidae				Janiridae
Walking preference	sideway	sideway	forward	forward	forward	forward	forward	forward
<b>Muscles</b>								
<b>Levators</b>	a (4)	a (4)	a (2-3) m (0-2)	a (3)	a (3)	a (1)	a (1)	a (1)
	p (2)	p (2)	p (1-3)	p (2)	p (1)	p (1)	p (3)	p (1)
<b>Depressors</b>	dep (4)	dep (4)	a ( 4-6) m ( 0-6) p (1-2)	dep (6)	a (1)	a (2)	a (2) m (2)	l (1) m (1-2)
			p (1-2)		p (4)	p (1)	p (1)	
<b>Promotors</b>	pro (2)	pro (2)	a (1-2) p (0-3)		a (1-3) p (0-2)	a (1) p (1)	pro (1)	pro (2-3)
<b>Remotors</b>	a (1) p (1)	a (1) p (1)	a (1-3) p (0-1)		a (1-3) p (0-2)	a (1) p (1)	rem (1)	rem (2)
<b>References</b>	<b>a</b>	<b>b</b>		<b>c</b>	<b>d</b>	<b>e</b>	<b>f</b>	<b>g</b>

Comparison between the muscle bundles comprising the proximal musculature of spider crabs (after Antonsen and Paul, 2000), and those of other decapods (as reported in the literature). The presence of multiple heads within a group is indicated by a letter followed by the number (or range) of muscle bundles described (in parenthesis). Empty cells indicate data not reported. <sup>a</sup> Bévengut et al., 1983; <sup>b</sup> Cochran, 1935; <sup>c</sup> Antonsen and Paul, 2000; <sup>d</sup> Pilgrim and Wiersma, 1963; <sup>e</sup> Macmillan, 1975; <sup>f,g</sup> Hessler, 1982.

**Table 3.4.** Comparison of musculature between the pereopods of *L. emarginata*.

<i>Muscle</i>	<i>Depressors</i>	<i>Levators</i>	<i>Promoters</i>	<i>Remotors</i>
<b>Pereopod</b>				
<b>1</b>	a (4), m (6), p (2)	a (3), m (2), p (1)	a (3)	a (1), p (3)
<b>2</b>	a (6), p (2)	a (2), p (3)	a (2), p (1)	a (1), p (1)
<b>3</b>	a (4), p (1)	a (3), p (2)	a (1), p (1)	a (1)
<b>4</b>	a (5), p (1)	a (3), m (2), p (1)	a (3)	a (2)
<b>5</b>	a (4), p (1)	a (2), m (2), p (1)	a (1)	a (2)

Numbers and locations of muscles and muscle heads for the proximal musculature of each pereopod. Anterior (a), medial (m), and posterior (p) muscles comprising a muscle group are followed by the number of muscle bundles that comprise them (in parenthesis).

Findlay and McVean, 1977; Moffett et al., 1987) The addition (or separation) of bundles in the two terminal limbs of *C. maenas* and *L. emarginata* hints to a need for further uncoupling of the autotomy and levation mechanisms in these limbs.

### **Coxal Muscles**

Key during forward walking is the musculature that moves the coxa and the entire leg in the forward and backward direction. Two muscle groups are responsible for this motion, the promoters and remotors, which differ from each other in number of bundles and in size. The promotor muscles tend to have multiple and distinct bundles which sometimes can be segregated into two muscles, as is the case in the second and third pereopods (Fig 3.4 and 5). In both of these legs a second (posterior) promotor muscle is present and inserts directly onto the dorsal lip of the coxa without the aid of an apodeme. The number of promotor heads might be important for the fine control of leg positioning during promotion in forward walking. Except in the first pereopod where a second muscle is present and inserts on an anterior apodeme (Fig 3.3G, H), the remotor musculature comprises a large and a smaller bundle. The remotor muscle

usually fully occupies the posterior dorsal compartment of the endosomite and its size is likely a consequence of the need for generating the propulsive forces required during forward locomotion.

### **Concluding Remarks**

We distinguished between muscle bundles on the basis of anatomical features alone. In other species, different bundles of proximal muscles were shown to be independently innervated (Antonsen and Paul, 2000) raising the possibility of for independent activation of the different bundles. In *L. emarginata* we have observed differential activation of proximal bundles (Chapter 5) for the coxal and thoracic bundles of the depressor muscles.

Much research has been conducted on the physiological properties of the crustacean muscles responsible for the production of legged locomotion (Atwood, 1963, 1977; Fatt and Katz, 1953; Günzel et al., 1993; Honsa and Govind, 2002; McDermott and Stephens, 1988; Tse et al., 1983). The wealth of neuroethology research that has been conducted on decapod crustaceans is based on the sometimes non-extensive anatomical knowledge of the machinery that is instrumental in the production of behavior. Only by elucidating this machinery and its properties can we hope to fully understand how adaptive behavior is produced. Differences in distal musculature between *L. emarginata* and other brachyurans support the idea that spider crabs show forward walking adaptations in their musculature that are present in other forward walking crustaceans. Although we cannot establish if these adaptations are analogous or homologous between these species, it is likely that they confer an important advantage to their owner for the production of forward locomotion. By fully elucidating the walking machinery in the forward walking spider crab and comparing it with that in sideways walking brachyurans, we can expect to better understand the nature of legged locomotion in general.

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## **CHAPTER 4**

### **MAP OF THE MOTOR NEURONS INNERVATING THE LEGS OF THE FORWARD WALKING CRAB, *LIBINIA EMARGINATA* (DECAPODA, BRACHYURA, MAJOIDEA)**

## INTRODUCTION

The behavioral repertoire and research amenability of decapod crustaceans has made them choice organisms for well over a century (for overview see Atwood, 1977; Hoyle, 1976; Schram, 1986). Reptantian (walking) species in particular have been subject to intense research on the production of legged locomotion (Macmillan, 1975; Ayers and Clarac, 1978; Clarac, 1977; Sillar and Skorupski, 1986; Jamon and Clarac, 1995; Martinez, 1996; Martinez et al., 1998; Cattaert and Le Ray, 2001). Much of what we now know about crustacean locomotion comes from studies on forward walking macrurans (Macmillan, 1975; Jamon and Clarac, 1995; Cattaert and Le Ray, 2001) or sideways walking brachyurans (Clarac et al., 1987; Martinez et al., 1998).

Neuroethologists often find themselves having to mix and match knowledge from different species with diverse ancestries or behavioral repertoires in order to arrive at a comprehensive description of the production of a behavior. The need to identify an organism that produces an interesting, comparable behavior and amenable to the many techniques used in neuroethology led us to select the portly spider crab, *L. emarginata*, as a subject.

*L. emarginata* is a brachyuran crab that walks preferentially forward 80% of the time (Schreiner, 2004). As a majoid, *L. emarginata* belongs to the group thought to have first evolved the crab form from lobster-like ancestors (Rice, 1983; Morrison et al., 2002). This possibility makes this brachyuran interesting for evolutionary studies as comparisons between spider crabs and other decapod crustaceans may shed light on the adaptations accompanying walking preference in decapod crustaceans.

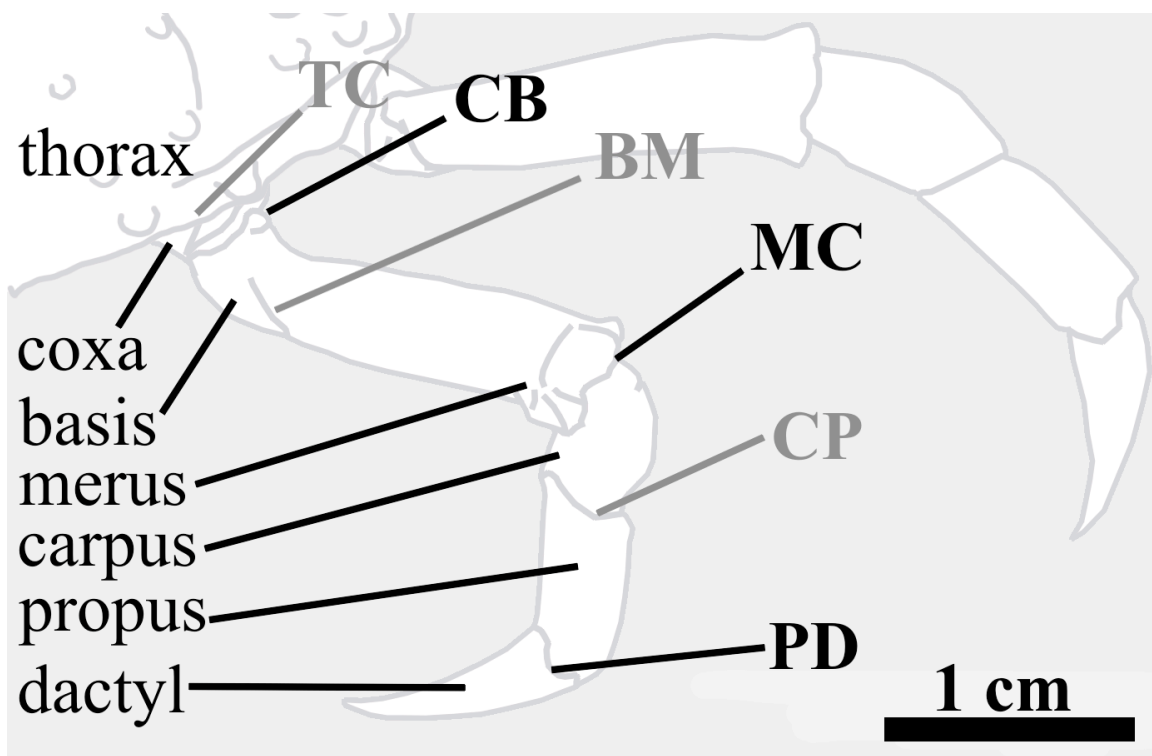
Previous work in our laboratory described the existence of skeletal and muscular adaptations for forward walking in spider crabs (Vidal-Gadea et al., 2008). Briefly, radially arranged pereopods having increased ranges of motion in joints responsible for forward-

locomotion, as well longer leg segments housing the musculature causing this motion were described for *L. emarginata* when compared with the sideway walking brachyuran *Carcinus maenas* (Vidal-Gadea et al., 2008). The musculature of *L. emarginata* also shares similarities with some forward walking crustaceans in that they have a large number of muscle heads for the proximal musculature (Chapter 3). The latter presents the possibility of some of these bundles being independently innervated and activated (as in the squat lobster; Antonsen and Paul, 2000).

Although the neuroanatomy of the segments distal to the plane of autotomy was shown to be highly conserved among decapods (Faulkes and Paul, 1997, Wiersma and Ripley, 1952), greater variability in the more numerous motor neurons innervating the proximal musculature was reported (Antonsen and Paul, 2001; Bévengut et al., 1983; El Manira et al., 1991a and b).

The typical brachyuran limb consists of six segments articulating at bicondylar joints that restrict motion to single planes perpendicular to the adjacent joints (Fig 4.1). With the exception of the reductor muscles of the basischiopodite that rotate the leg axis forward, the joints and musculature of brachyurans can be grouped into those that propel the animal sideways (producing adduction and abduction), and forward and backward (producing promotion and remotion, Fig 4.1). During locomotion, crustaceans walking sideways rely heavily on the distal MC joint (merus-carpus, see Table 4.1 for abbreviations used) while they use primarily their proximal joints (TC, CB) while walking forward (Ayers and Clarac, 1978).

We conducted this study to provide an anatomical framework in which to base future neuroethological studies of locomotion in spider crabs. We compare the neural anatomy of a forward walking brachyuran to that described for sideway walking brachyurans and forward walking macrurans.



**Figure 4.1.** The segments and joints composing the legs of spider crabs. The six segments that make up the legs of *L. emarginata* articulate with each other via bicondylar joints that restrict movement to the sideways direction (black), of the forward direction (gray).

**Table 4.1.** Abbreviations used throughout this work.

<b>aFE</b>	Accessory flexor excitor motor neuron
<b>BE<math>\alpha</math></b>	Anterior bender excitor motor neuron
<b>BE<math>\beta</math></b>	Posterior bender excitor motor neuron
<b>BM</b>	Basischiopodite-meropodite joint
<b>CB</b>	Coxopodite-basipodite joint
<b>CE</b>	Closer excitor motor neuron
<b>CI</b>	Common inhibitor motor neuron
<b>CP</b>	Carpopodite-propodite joint
<b>EE</b>	Extensor excitor motor neuron
<b>IGP</b>	Inter-ganglionic processes
<b>LTN1-8</b>	Left thoracic neuromeres one through eight
<b>MC</b>	Meropodite-carpopodite joint
<b>OI</b>	Opener inhibitor
<b>Op=Str</b>	Opener and stretcher excitatory motor neuron
<b>PD</b>	Propodite-dactylopodite joint
<b>RE<math>\alpha</math></b>	Anterior remotor excitor motor neuron
<b>RE<math>\beta</math></b>	Posterior remotor excitor motor neuron
<b>RTN1-8</b>	Right thoracic neuromeres one through eight
<b>SI</b>	Stretcher inhibitor
<b>TC</b>	Thorax-coxopodite joint
<b>TG</b>	Thoracic ganglia

## **MATERIALS AND METHODS**

### **Species Used**

*Libinia emarginata* crabs (n>100) were obtained from the Marine Resources Center of the Marine Biological Laboratories in Woods Hole, Massachusetts. Crabs were kept at 20°C in artificial seawater and fed a diet of catfish and dry pellets until used. We used animals ranging in size from small (length=3.5 cm) to large (length>10 cm).

## **Dissections**

Animals were cold anaesthetized and euthanized before each dissection. Prior to dissection, the preparations were immersed in 4°C crab saline (Rathmayer and Erxleben, 1983) with 1% methylene blue to stain the leg nerves. Methylene blue was added with a pipette to freshly dissected areas. We performed ventral dissections by removing the abdomen and ventral skeleton, which exposed the thoracic ganglia (TG) and endophragmal skeleton housing the proximal musculature. Because in *L. emarginata* the TG is not occluded by leg musculature, we were able to access the TG and proximal musculature ventrally without disturbing either system.

## **Physiological Recordings**

Hook electrodes were mounted proximally on leg nerves for stimulation of individual nerves. An en passant suction electrode was then placed distal to the stimulating electrode to record motor activity. A pair of silver wire electrodes was inserted in the muscle of interest for EMG confirmation of the motor nerves activated during stimulation. Signals were passed through an A-M Systems 1700 amplifier with 100-5000Hz filtering. The data was fed into a computer with Datapac 2K2® software for storage and analysis. Datapac 2K2® was set to a 10,000Hz sampling rate for all recordings. Stimuli were administered using an A-M Systems stimulator. The stimuli consisted of 0.1ms pulses of increasing amplitude and/or frequency. We confirmed the identity of the nerves stimulated by observing both the motion produced in the limb following stimulation and the EMG activity in the muscle. If a nerve innervated multiple muscles, a glass electrode was used to segregate the different populations of axons and to proceed with the stimulation and retrograde staining. We only backfilled nerves for which we had visual confirmation through produced joint motion of the muscle in innervated.

### **Retrograde Staining**

Once a nerve containing a motor neuron population of interest was identified, the nerve was cut distally, removed with the TG still attached and backfilled using  $\text{CoCl}_2$  (Pitman et al., 1972), or  $\text{CoCl}_2$  and  $\text{NiCl}_2 + \text{CoCl}_2$  for dual color labeling (Quicke and Brace, 1979). After processing, the ganglia were dehydrated in alcohol series, cleared in methyl salicylate and mounted on Sylgard®-lined glass petri dishes.

### **Image Capture and Manipulation**

Images of backfills were captured using a camera-mounted Leica dissecting stereomicroscope. Images were adjusted for brightness and contrast using CorelDRAW®. Photographs obtained by focusing at different depths though the backfills were traced on CorelDRAW® to combine different focal planes or partial backfills. This was particularly useful since the TG of *L. emarginata* can be deep and therefore difficult to image in whole mount.

## **RESULTS**

### **Thoracic Nervous System and Ganglia Morphology**

Ventral dissection of the skeleton of *L. emarginata* revealed the thoracic ganglia (TG) to be located medially and anteriorly in the thorax. Because *L. emarginata* is not laterally compressed ventro-medially, the ganglia lie in close apposition with the ventral skeleton and removed from the musculature that occludes them in other decapods that are laterally compressed. The location of TG allows direct ventral access to it without disturbing the leg musculature. Although in smaller specimens (3-3.5 cm long) the ganglia are located centrally within the thorax, in larger animals (10-12 cm long) they are found more anteriorly. Consequently, most of the leg nerves travel posteriorly a considerable distance to the muscles they serve. The layout of the nervous system of *L. emarginata* allowed us to stimulate and record from the exposed nerves without disturbing the leg musculature.



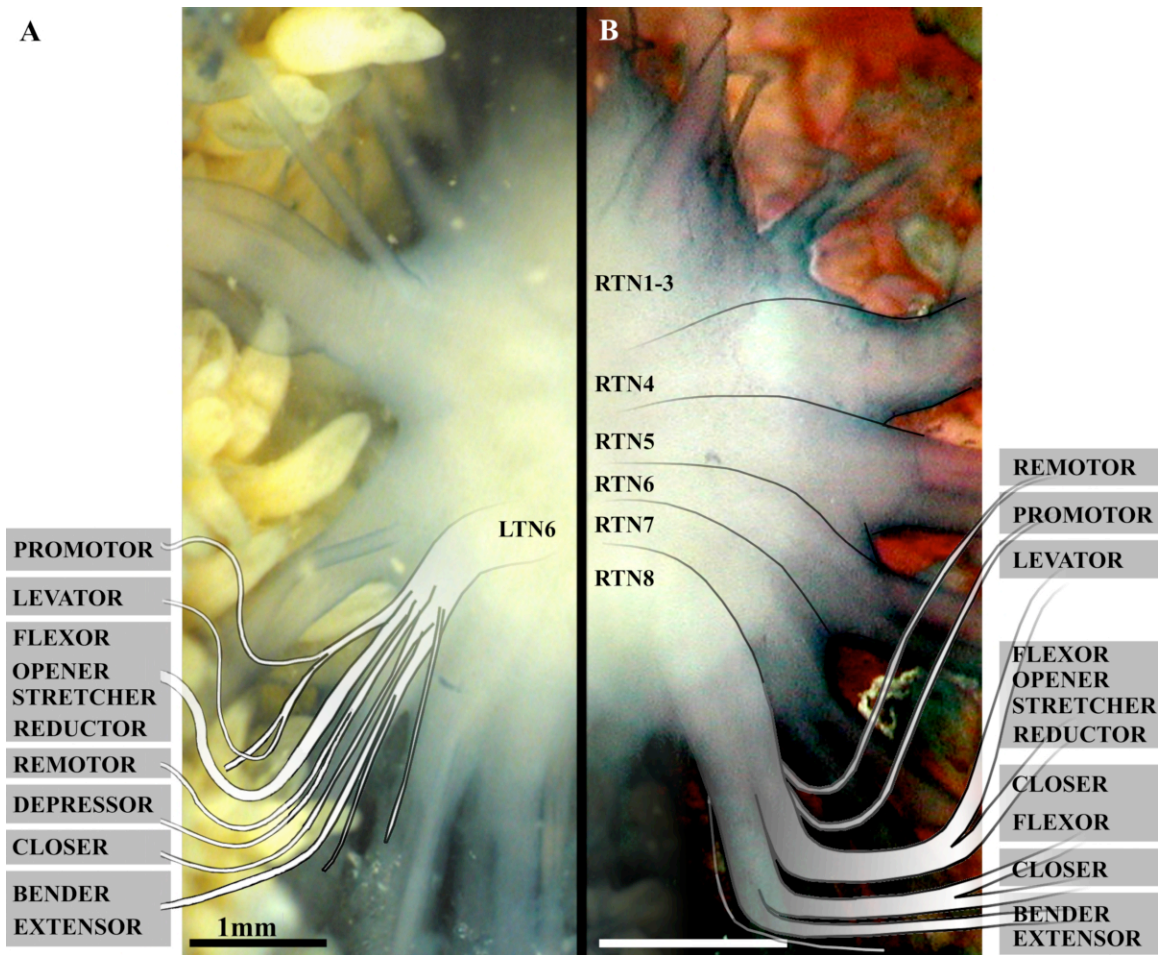
The TG of *L. emarginata* consists of the fused thoracic neuromeres, one through eight, (TN1-8) of which the fourth and eighth neuromeres are largest. The fused abdominal pleomeres partially overlies the last pair of thoracic neuromeres. The thoracic sternal artery that bisects the TG of *Procambarus clarkii*, *Callinectes sapidus* and *Carcinus maenas* does not traverse *L. emarginata*'s. Instead, the sternal artery covers the dorsal surface of the ganglia and bifurcates into smaller vessels that follow the leg nerves into the pereopods. Mirroring the radial arrangements of the pereopods around the thorax, the ganglia also assume a radial orientation as one moves posteriorly (Fig 4.2). The difference in neuromere orientation becomes clear when observing the position of homologous motor neurons in different segments.

### **Motor Nerves**

The exit routes of the leg motor neurons follow a similar plan to that described for *P. clarkii* (see Cattaert and Le Ray, 2001) and *C. maenas* (Bévengut et al., 1983), but we saw variability between specimens (Fig 4.2). This led us to use physiological methods to confirm the identity of the motor neurons contained in any particular nerve. Stimulation of the different nerves exiting the ganglia revealed that the location of motor nerves varied between large and small specimens and between anterior and posterior neuromeres (Fig 4.2). The number of individual nerves arising from each neuromere is larger in large animals and for posterior pereopods.

### **Distal Innervation**

A total of 19 somata were backfilled from nerves of the distal musculature of the legs of *L. emarginata*. We will describe the location of the motor neurons innervating the distal leg musculature and for practical reasons chose to group this description by the exit routes of the motor neurons rather than by their target muscles.



**Figure 4.2.** Comparison between the thoracic ganglia of a medium (A), and a small (B) sized *L. emarginata* showing examples of the exit routes of motor axons to the walking legs. The fifth through sixth neuromeres are oriented more posteriorly in the larger specimen owing to the more anterior location of TG in larger specimens. Ventral view, anterior is up.

### **Closer Excitors**

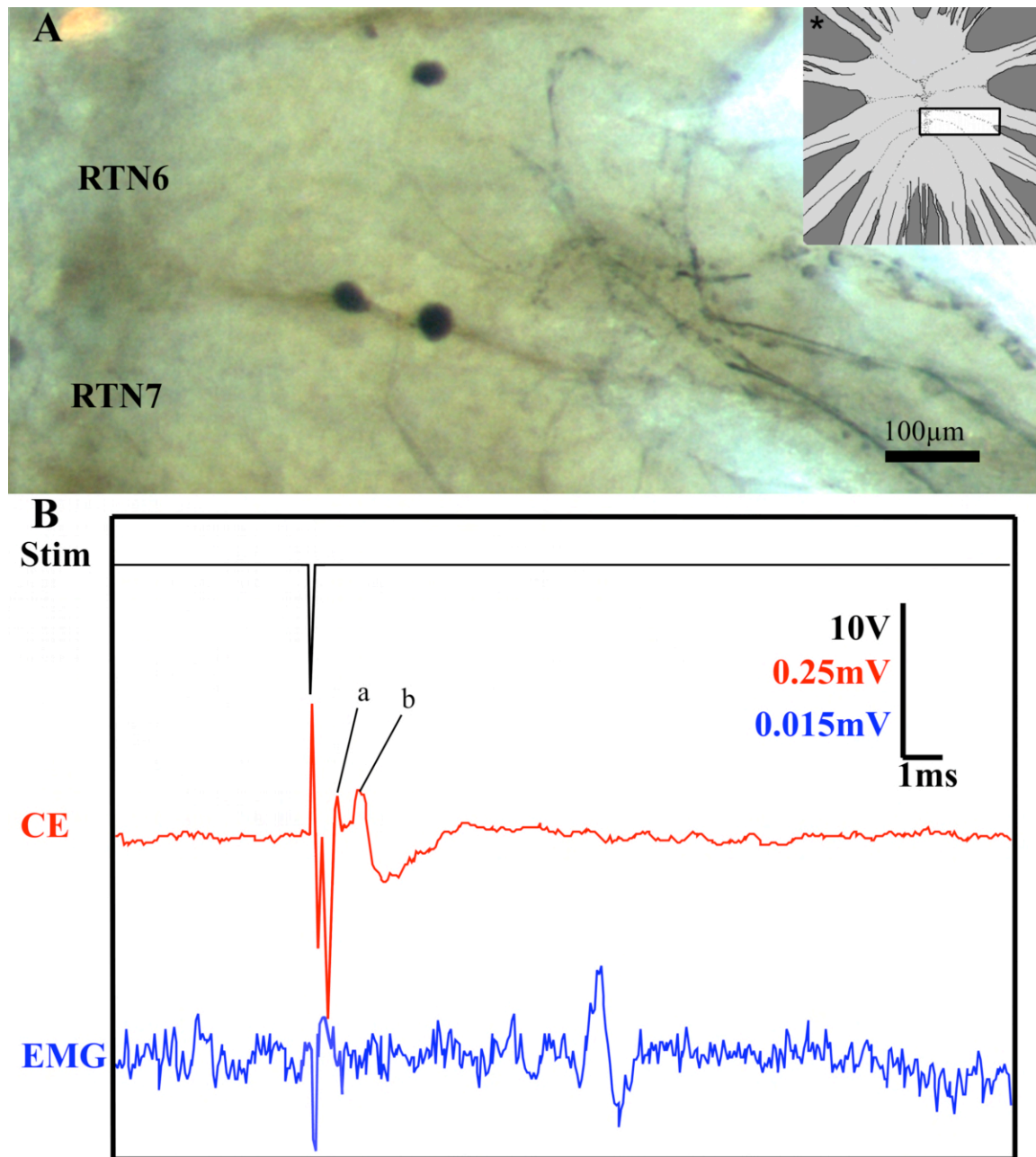
The closer muscle of *L. emarginata*, responsible for flexion of the dactyl at the PD joint, is innervated by two excitatory motor neurons (Fig 4.3A) and by the common inhibitor neuron (CI). In *L. emarginata*, as in all other species that have been examined, the CI innervates every muscle in the leg. Backfills of the closer nerve yielded two closely associated somata located dorsally on the medial anterior surface of the ganglion. These neurons send their processes a short distance ventrally and then turn posteriorly converging into a common track that turns distally and exits along a private nerve. The number of somata was confirmed by physiological recordings from this nerve (Fig 4.3B).

### **Bender Excitors**

The bender muscle is responsible for flexion of the propodite in the anterior direction and, with the exception of the first pereopod, consists of a single muscle. It is innervated by two excitatory neurons and by the common inhibitor (Fig 4.4A, C, D). The two bender excitors lie dorsally on the ganglion but differ otherwise in location. The most anterior bender excitor ( $BE\alpha$  nomenclature of Faulkes and Paul, 1997) lies anteriorly on the medial half of the ganglion. The second bender ( $BE\beta$ ) lies posteriorly on the ganglion and distal to the  $BE\alpha$ .

### **Extensor Excitors**

The extensor muscle is responsible for extending the carpopodite at the merus-carpus joint. There are two excitor cells for this muscle and they usually travel in the same nerve as the bender, often necessitating segregation with a glass microelectrode to separate the two groups. These two cells lie dorsally in the ganglion (Fig 4.4B-D) and are distal to the closer excitors already mentioned. The most medial of these cells is slightly larger and more anterior than its counterpart.



**Figure 4.3.** **A)** Closer excitor somata of the right fifth and sixth thoracic neuromeres (\* shows location in the TG). **B)** Recording from the closer excitor neurons (CE), and the closer muscle (EMG) confirming the presence of two different (**a** and **b**) cells in the closer nerve.

## **Flexor Excitators**

The flexor musculature of *L. emarginata* is responsible for the flexion of the merus-carpus joint. The flexor motor neurons exit the ganglia and enter the leg in the same nerve as the reductor excitators and the single opener=stretcher excitators (Fig 4.1). Backfills of this nerve revealed the presence of seven different cells all of which were found dorsally in the ganglion with seven clustering anteriorly on the middle of the ganglionic surface (Fig 4.5A, B).

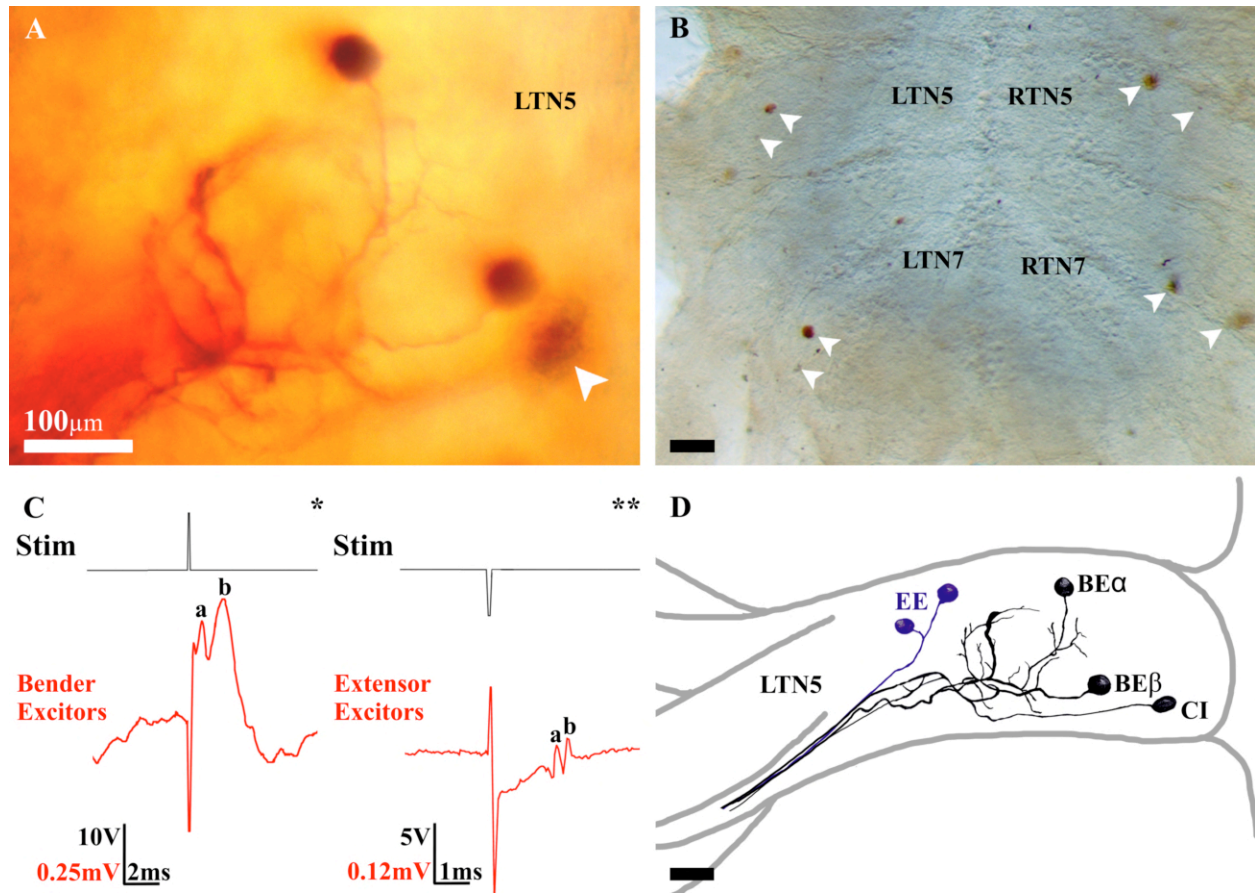
In addition to the somata backfilled, several inter-ganglionic processes (IGP) were also consistently filled in flexor preparations. Several of these axons originated from cells lying medially (both ipsilaterally and contralaterally) in the anterior neuromeres (Fig 4.5D, F).

## **Reductor Excitators**

Two reductor muscles within the basischiopodite are responsible for pitching the leg axis forward at the basis-merus joint. Two dorsal excitatory motor neurons were found to innervate these muscles. The most anterior of these cells ( $RE\alpha$ ) lies medially on the ganglia just distal to the flexor excitators, and the second reductor excitator soma ( $RE\beta$ ) is located dorsally on the center of the ganglia (Fig 4.5C). The axons of both cells converge medially and distally before joining the bender excitators on their way to the end part of the pereopod.

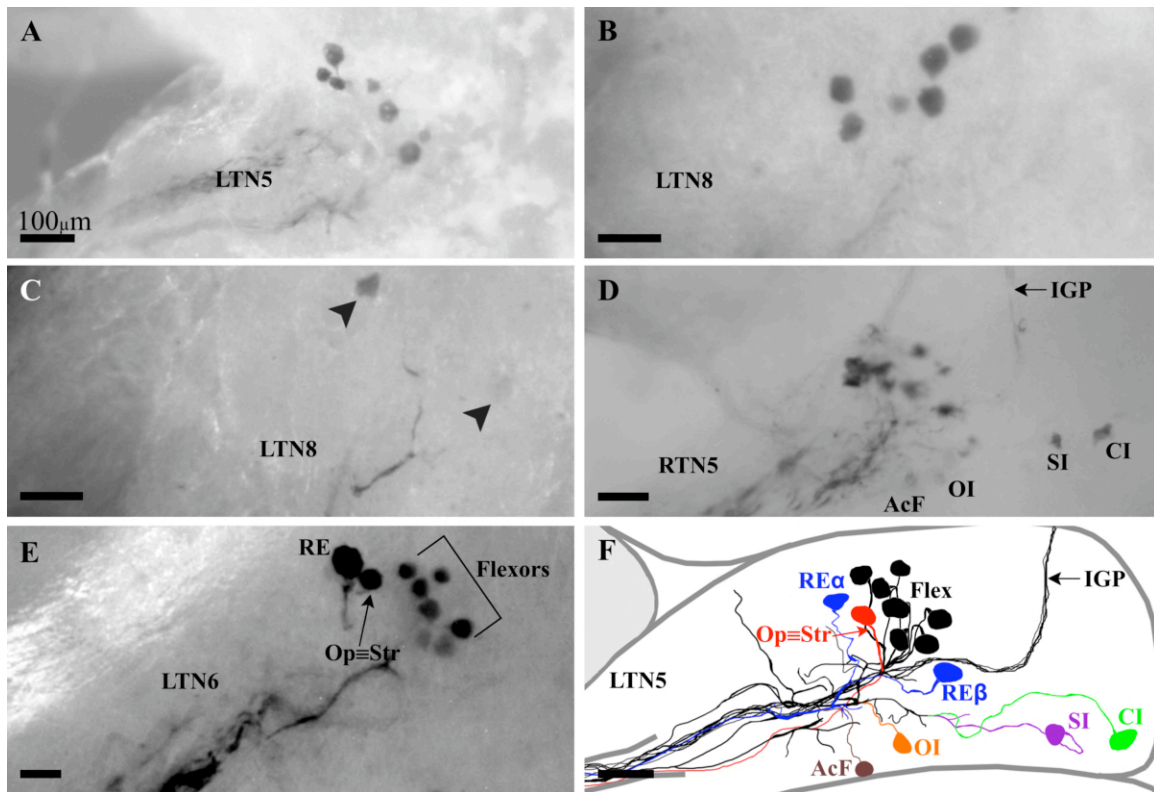
## **Accessory Stretcher and Additional Somata**

Besides the anteriorly located somata described above, we were also able to stain four additional somata posteriorly on the ganglion (Fig 4.5D, F). Based on the similar location of these cells to the ones described in previous work on other decapod crustaceans (Faulkes and Paul, 1997), we believe these to be the somata of the common inhibitor (CI). The CI is located on the posterior medial corner of the ganglion and deeper than the excitatory somata. Closely associated with CI, but more dorsal and distal, a second cell was stained in the same location as the previously described Stretcher Inhibitor (SI, Wiens and Wolf, 1993). A third cell also lying



**Figure 4.4.** Dorsal view of retrograde stainings showing the bender and extensor excitators. **A)** Two bender excitators ( $BE\alpha$  and  $BE\beta$ ), were stained with  $NiCl_2+CoCl_2$  for precipitation with Rubenic acid. The white arrow head shows the position of the common inhibitor neuron. The two extensor excitators (EE) often leave the ganglia in the same nerve root as the bender excitators. Anterior is up and medial is left. **B)** Backfill with  $CoCl_2$  showing the pair of extensor excitators (arrow heads) innervating the extensor muscle of the second and fourth pair of pereopods. Anterior is up. **C)** Physiological recordings confirming the number of cells innervating the bender (\*), and extensor (\*\*) musculature. **D)** Diagram showing the relative position of the benders, extensors, and the common inhibitory neurons within the ganglion. Dorsal view, anterior is up and medial is right.





**Figure 4.5.** Retrograde staining showing the number and location of flexor, reductor, and opener-stretcher motor neurons. Backfilling the flexor nerve revealed seven motor neurons clustered anterior dorsally on the thoracic neuromeres of *L. emarginata*, here shown for the left second (A) and fifth (B) walking legs. Note the rotation of the cluster due to the radial arrangement of the ganglia. C) Location of the two reductor excitor motor neurons (arrow heads). D) Ventral view of a flexor stain showing four somata tentatively labeled as the Closer Inhibitor (CI), Stretcher inhibitor (SI), Opener inhibitor (OI), and Accessory flexor (AcF) based on previous work on related species (Faulkes and Paul, 1997). Most flexor backfills included a set of inter-ganglionic processes (IGP) that traveled to the midline and then turned anteriorly to their origin is anterior ganglia. E) Backfill showing the location of the opener and stretcher excitor (adjacent to the anterior reductor excitor). F) Diagram showing the relative positions of the motor neurons labeled. Anterior is up and proximal is left. All views are dorsal except in D.

posteriorly was found more ventrally than the others and behind the flexor cluster. This is likely the opener inhibitor (OI). The most distal of the four posterior somata lies adjacently to OI although not as ventrally and is likely the accessory flexor soma and has also been previously described for related species (Faulkes and Paul, 1997).

### **Opener=Stretcher Excitators**

The opener muscle is responsible for extension of the dactyl and shares a single excitatory innervation with the stretcher muscle (responsible for extension of the propodite). By segregating the opener excitatory motor axon and backfilling the remaining nerve, we were able to demonstrate this neuron (Op=Str) to be adjacent to the flexor excitators and between the two reductor somata (Fig 4.5E).

### **Proximal Innervation**

Retrograde staining of the nerves serving the proximal musculature revealed 64 motor somata lying near the surface of the ganglia.

### **Levators**

The levator muscles of *L. emarginata* are located in the thorax and the coxa and elevate the leg off the substrate as well as being involved in limb autotomy (McVean and Findlay, 1976). A total of 16 cells were backfilled from the nerve serving the levator musculature (Fig 4.6). The somata were clustered in three groups, two groups lying dorsally on the anterior-distal surface of the ganglion, and a smaller group lying deeper on the posterior distal surface of the ganglion. Of the two anterior groups, the most medial consists of five cells, and the distal of eight cells. The axons of each anterior group travel posteriorly and then converge before turning in the distal direction to form a common track on their way to the musculature they innervate. Axons from the posterior somata cluster also converge on this track.



As described above for the flexor backfills, the levator backfills also stained inter-ganglionic processes that turned anteriorly after exiting the ganglia medially (Fig 4.6C,D).

### **Depressors**

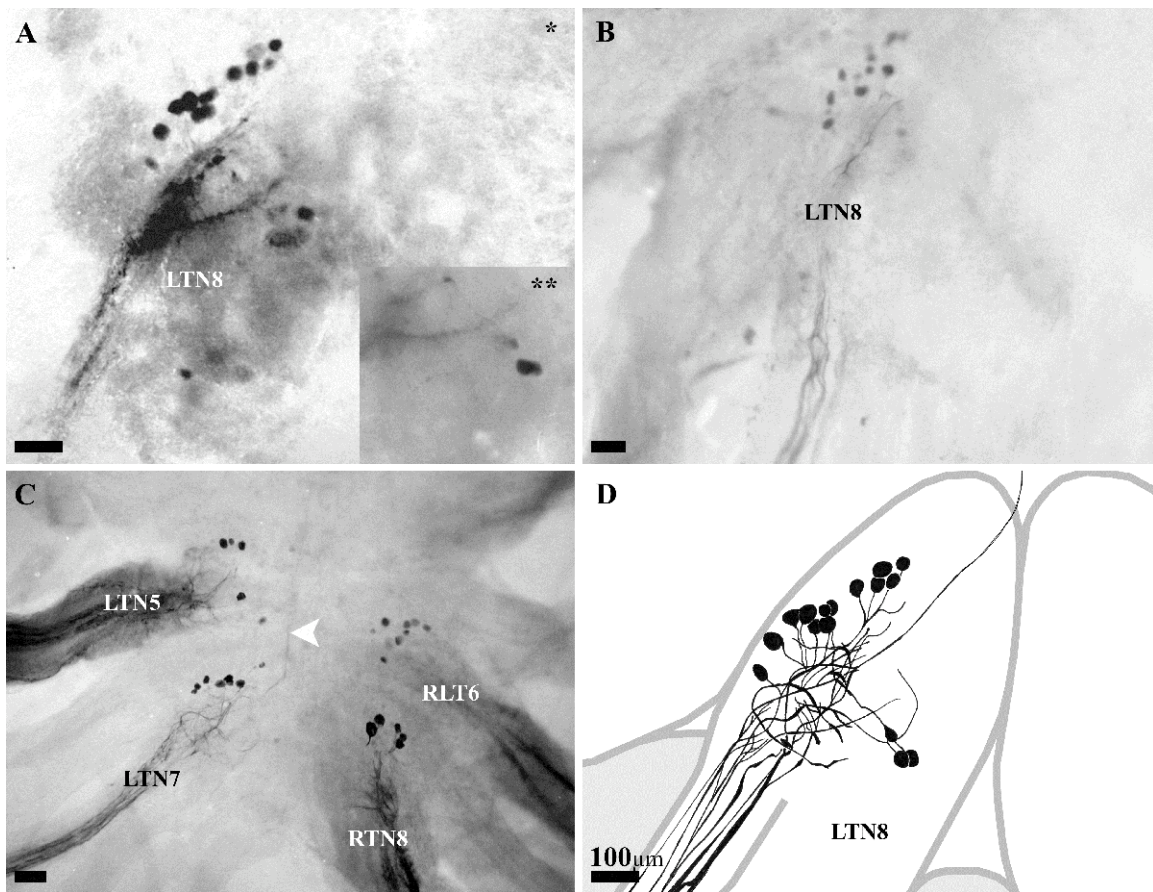
The depressor musculature is responsible for lifting the animal off the substrate and thus plays an important role in locomotion as well as in posture. We backfilled a maximum of 20 somata innervating this musculature (Fig 4.7). A group of ten tightly clustered motor neurons are located medially on the ventral-posterior surface of the ganglion. The remaining somata are all found dorsally with one cell lying more laterally and anteriorly and another one centered on the dorsal ganglionic surface. The last eight somata were all anterior and dorsal and medial to the ventral cluster.

### **Promotors**

The promotor musculature is responsible for promotion of the leg in the anterior direction and consists of at most three muscle heads. We found that 17 cells were labeled by backfilling the promotor nerve (Fig 4.8). Fifteen of the somata are dorsal and two are ventral. The dorsal somata are clustered in two groups. The largest group consists of thirteen cells located anteriorly and proximally on the ganglion. The smallest dorsal cluster is composed of two closely associated cells which are centered below the dorsal ganglionic surface, and a third cell located more medially. The remaining two cells are just medial to the latter group on the ventral surface of the ganglia. The axons of the promotor motor neurons form a common track that extends distally towards the leg and is located anterior to the levator track described above.

### **Remotors**

The remotor musculature is responsible for moving the leg in the posterior direction and consists, depending on the pereopod, of one to three muscle heads. Backfills of the remotor nerve



**Figure 4.6.** Retrograde stainings showing the location and numbers of motor neurons innervating the levator musculature of spider crabs. **A and B)** Examples of left eighth neuromeres showing the anterior location of 13 levator somata. **C)** partial backfills of the levators of the last four thoracic neuromeres showing the radial arrangement of the ganglia. The inter-ganglionic processes observed in the flexor backfills were also observed in levator backfills (arrow head). **D)** Diagram of the 16 motor neurons filled through the levator nerve. Anterior is up and proximal is right (except in **C**).

revealed 11 somata (Fig 4.9). A tightly clustered group of nine cells is located mid-posteriorly on the ventral surface of the ganglion close to the midline. Additionally, one cell is located more distally and anteriorly on the ganglia, and a second backfilled cell is posterior to those of the main cluster.

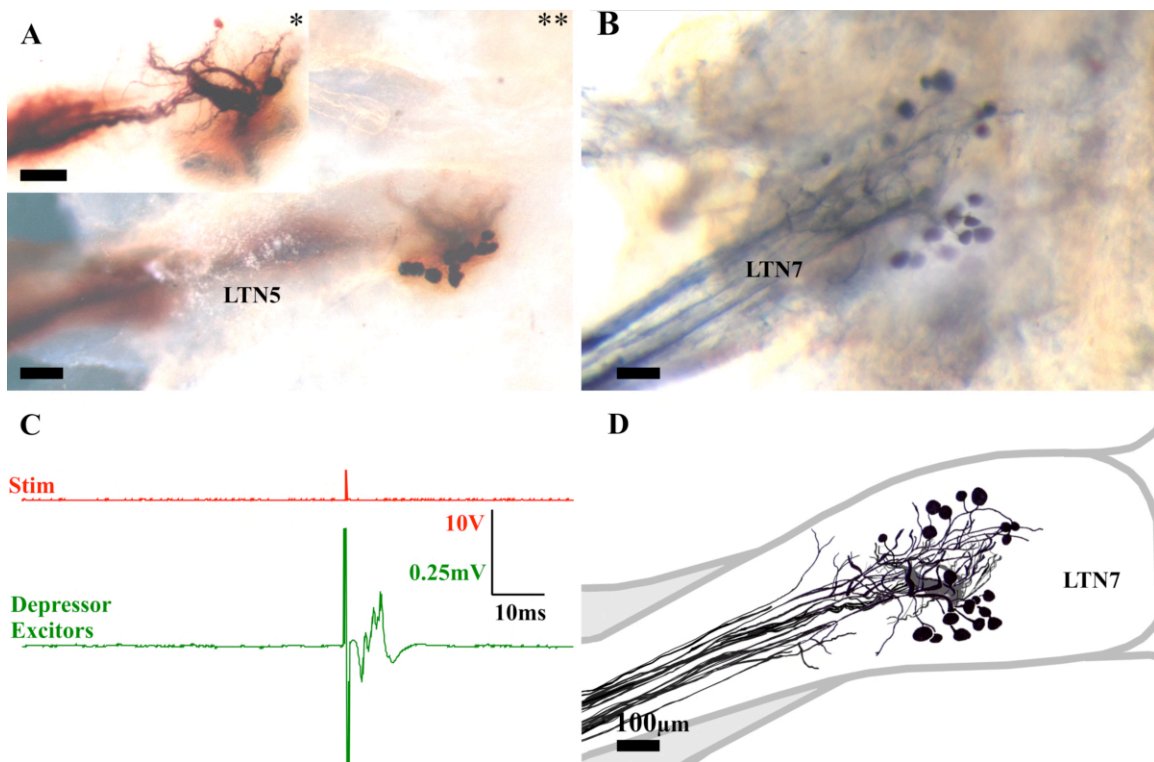
In addition to the somata described above, backfills of the remotors of the eighth neuromere often revealed some additional somata that were adjacent to those of the contralateral remotor group (not shown). A pair of bilaterally symmetrical somata close to the midline was backfilled. This sends extensive processes to both eighth neuromeres before joining the remotor tracks of each pereopod on their way to the periphery. Review of the literature, however, suggests these may be motor neurons belonging to the abdominal ganglia, which are fused with the last thoracic neuromeres and not remotors excitators of the eighth neuromeres (Paul et al., 1985; Paul and Wilson, 1994; Paul and Bruner, 1999).

## **DISCUSSION**

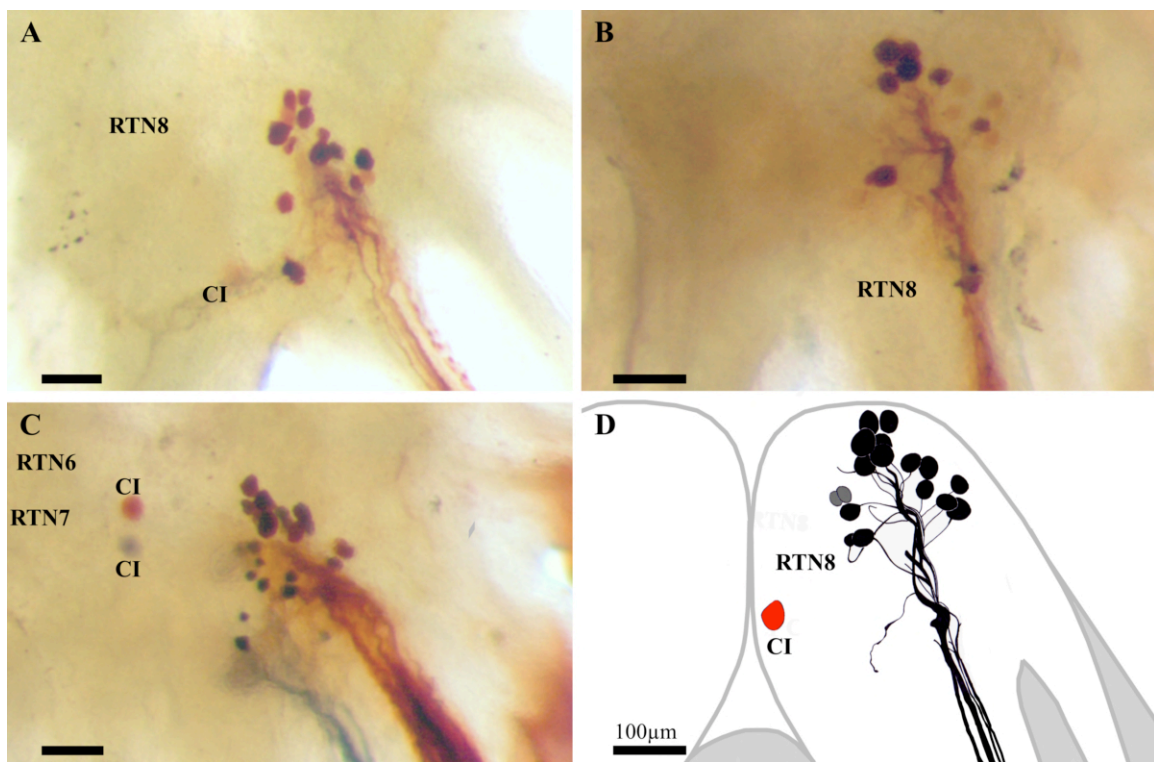
### **Thoracic Ganglia**

We found the thoracic nervous system of *L. emarginata* to conform loosely to the established crustacean plan (Wiersma and Ripley, 1952; Bévengut et al., 1983; Elson, 1996; Cattaert and Le Ray, 2001). Variability in axonal exit routes observed (Fig 4.2) was reported for other species (Faulkes and Paul, 1997) and in *L. emarginata* is likely the product of capricious defasciculation during development. As the animal increases in size, the thoracic ganglia become more removed from the musculature they innervate and motor axons previously grouped together may become separated from each other. These effects could be magnified in *L. emarginata* due to the lack of ventral skeletal compression in this species (Vidal-Gadea et al., 2008).

We observed occasional differences in the relative orientation of the neuromeres with some being slightly rotated compared to its contralateral and ipsilateral neighbors. These



**Figure 4.7.** Motor neurons innervating the depressor musculature of *L. emarginata*. **A)** Dorsal (\*) and ventral (\*\*) views of a partial backfill of the depressor motor neurons of the left fifth thoracic neuromere showing the extensive integrating zone in the center of the ganglion. **B)** Composite showing a backfill of the complete depressor motor neuron complement innervating the left fourth walking leg of *Libinia*. **C)** Physiological recording confirming the identity of the nerve serving the depressor. **D)** Diagram showing the position of the depressor somata in the ganglia. Anterior is up and proximal is right.



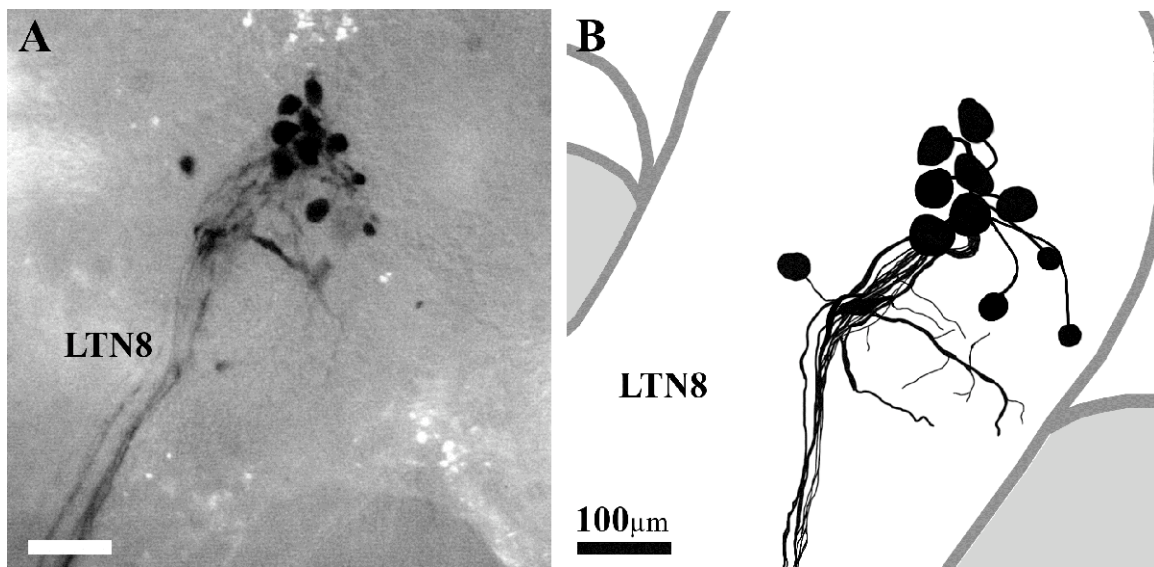
**Figure 4.8.** Motor neurons innervating the promotor musculature of *L. emarginata*. **A)** Dorsal view of a  $\text{NiCl}_2+\text{CoCl}_2$  backfilled promotor nerve showing the 15 dorsal somata and the common inhibitor (CI) of the last right thoracic neuromere. **B)** Partial backfill of the same promotor population as in **A**. **C)** Differential backfill of the promotor population of the right sixth neuromere (with  $\text{NiCl}_2+\text{CoCl}_2$ : red) and the seventh neuromere ( $\text{NiCl}_2$ : blue) showing the variability in somata size between the two neuromeres. The common inhibitor (CI) is visible in both backfills. **D)** Diagram of the last thoracic ganglia showing the location of the promotor somata, the two ventral somata not seen in dorsal view (for a total of 17 promotor neurons) are shaded gray, and the common inhibitor is colored red. Anterior is up and proximal is left. All views are dorsal.

differences from other described decapods may be partially explained by considering that neurogenesis continues well into the zoeal stages in spider crabs, whereas it is over before metamorphosis in macrurans (Harzsch et al., 1998).

The fourth and eighth thoracic neuromeres are larger than the rest. These differences in size between ganglia are possibly due to the larger neuropillar size correlated with the larger behavioral repertoire for these two legs (Mulloney et al., 2003). The fourth thoracic neuromeres control the claws. The eighth neuromeres are responsible for the righting behavior of overturned crabs and also possess a larger behavioral repertoire than the anterior pereopods (personal observation).

### **Motor Neuron Pools**

The layout within the ganglia of the motor pools resembles that of related species (Bévengut et al., 1983). Most of the somata are located on the dorsal surface of the ganglia and are segregated into specific regions according to their function. As previously described for other crustaceans: the levator neurons congregate on the anterior lateral surface of the ganglia, the promotors are located anterior and medial, the depressors are posterior and lateral, and the remotors are posterior and medial (Bévengut et al., 1983). The central portion of the thoracic neuromeres is reserved for neuropils. Previous work revealed between 51 and 81 motor neurons innervating the leg musculature of decapod crustaceans (for review see Faulkes and Paul, 1997). We identified 82 potential motor neurons innervating the legs of *Libinia emarginata*. The possibility remains that some of the cells backfilled in our studies were in fact sensory or secretory in nature (Paul and Bruner, 1999; Paul and Wilson, 1994). Alternatively, motor axons serving different muscles could have been inadvertently stained if they failed to elicit muscular activity upon stimulation. An example of this caveat was evident in backfills of the remotors of



**Figure 4.9.** Retrograde staining of the remotor nerve labeled a total of 11 somata on the ventral ganglionic surface. **A)** Backfill of the left eighth thoracic neuromere showing the population of remotor motor neurons. **B)** Diagram showing the position of the remotor neurons within the ganglia. All views are ventral. Anterior is up, and medial is right.



the eighth neuromeres. Due to the radial arrangement of the legs in *L. emarginata*, the remotor nerves must extend quite far posteriorly to reach their target. In some preparations, the abdominal flexor inhibitors (described for *Munida quadrispina* by Paul et al., 1985) were backfilled along with the remotor nerve of the eighth neuromere. These cells were easily recognized due to their unique morphology and contralateral projections. A few additional somata were also labeled that were in all likelihood secretory in nature (see below) and could contribute to inflated somata numbers. The spatial segregation of functionally related somata within the ganglia minimized the risk of misidentifying cells. Whenever non-motor cells were identified, we left them out of our count for the motor pool under study.

Notwithstanding the possibility of labeling non-motor cells, the proximal musculature of *L. emarginata* is innervated by a considerably larger pool of motor neurons than that found in *C. maenas* and other sideways walking brachyurans. Generally, the number of motor neurons was larger in populations innervating muscles with more muscle heads. The depressor musculature, for example, received innervation from a total of 20 motor cells (Fig 4.7) and has also the largest number of muscle heads of any leg muscle in *L. emarginata* (Chapter 3).

### **Motor Neurons Innervating the Distal Musculature**

The number of motor neurons innervating the distal musculature of crustaceans described in the literature is fairly conserved and ranges between 14 in the American lobster (*Homarus americanus*; Wilson and Sherman, 1975), 16 in the spiny lobster (*Jasus novaehollandiae*; Silvey, 1981), and 17 in the squat lobster (*Munida quadrispina*, Faulkes and Paul, 1997). We backfilled up to eighteen somata innervating the distal musculature of *Libinia emarginata*, with the additional somata being associated with the flexor cluster (see below).



### **Closer Excitators**

The number and location of the neurons innervating the closer musculature closely resemble those described for several crustaceans (Faulkes and Paul, 1997; Govind and Lang, 1981; Wiersma and Ripley, 1952; Wilson and Mellon, 1982).

### **Bender and Extensor Excitators**

The bender and extensor excitators exit the ganglia in a common nerve. Separating the two nerves revealed a pair of bender excitators lying in a similar location to those described for other decapod species by Faulkes and Paul (1997). The two extensor excitators also were located on the anterior and distal surface of the ganglia as described for the crayfish (Bradac et al., 1996).

### **Opener=Stretcher Excitators**

Segregating the opener=stretcher axon from the flexor nerve allowed us to selectively include or exclude the opener=stretcher excitator from our backfills. This revealed a single soma in close association with the anterior reductor excitator ( $RE\alpha$ ) and just distal to the flexor somata (Fig 4.4C). This location is the same as previously described for this motor neuron in related species (Faulkes and Paul, 1997).

### **Flexor Excitators**

Previous work on *C. maenas* (Parsons, 1982) demonstrated the departure of at least this species from the accepted distal leg innervation plan described by Wiersma and Ripley (1952). Backfills of the nerve innervating the flexor muscle in *L. emarginata* legs revealed a group of seven closely associated somata (Fig 4.5). The flexor muscle of the legs of *L. emarginata* differs from that of *C. maenas* in having additional muscle heads of which one is bi-segmental (Chapter 3). The additional somata seen for the flexor muscle could be serving these additional muscle heads. We did not ascertain the nature of the cells innervating the flexor musculature as physiological properties of the motor neurons were beyond the scope of this study.

### **Accessory Flexor Excitor**

Our methodology did not permit us to discriminate between the flexor and accessory flexor muscles. Based on previous work (Faulkes and Paul, 1997) we inferred the identity of the accessory flexor somata to be the cell located posterior and distally on the ganglia (Fig 4.4D and F). Additional innervation of the accessory flexor muscle was revealed in several decapod species (Govind and Wiens, 1985). It is possible that one (or several) of the neurons backfilled in our experiment supply this muscle in lieu of the main flexor. This is however unlikely, based on previous work on the accessory flexor innervation of *L. emarginata* (Govind and Wiens, 1985).

### **Reductor Excitors**

Two distinct reductor muscles attach to the basipodite of *L. emarginata*. Work on related species identified two reductor excitor neurons that innervate them (Faulkes and Paul, 1997). Based on our axonal segregation experiments of the nerve carrying the flexors, reducers, opener=stretcher, and distal inhibitory neurons, two large somata were identified as the reductor excitors (Fig 4.4E). The reductor somata lie in close proximity to the flexor somata. This relationship echoes that of the musculature where the reductor apodeme serve as attachment site for a bi-segmental muscle head originating on the flexor apodeme (Chapter 3).

### **Inhibitors**

#### Common Inhibitor

The common inhibitor neuron, previously shown to innervate the entire musculature of the leg in brachyurans (Rathmayer and Bévengut, 1986; Wiens et al., 1988), was repeatedly labeled and presented no difficulty in identification. The CI of *L. emarginata*, as in other species, lies posterior and medial in the ganglia. In *L. emarginata* however, the CI is ipsilateral (Fig 4.4D, F), unlike the contralateral location described for it in *M. quadrispina* (Faulkes and Paul, 1997).

### Opener and Stretcher Inhibitors

Based on work carried on other species (Wiens and Atwood, 1978; Wiens and Wolf, 1993; Faulkes and Paul, 1997), we inferred the identities of the opener and stretcher inhibitors backfilled from nerves serving the distal musculature.

### **Motor Neurons Innervating the Proximal Musculature**

Previous work showed the motor neuron pools innervating the proximal musculature to vary more than that of the distal muscles (Antonsen and Paul, 2000). Unlike the distal musculature, the proximal muscles are housed in the thorax and consist of multiple muscle bundles capable of performing different behaviors. A classic example of this is the levator musculature, which consists of different muscles responsible for walking and autotomy of the limb (McVean and Findlay, 1976). The levator musculature was shown to receive at least partially independent innervation by levator motor neurons (Moffett et al., 1987).

### **Levator Excitors**

As described above, the role of the levator musculature and its innervation has received particular attention in relation to the process of leg autotomy (McVean and Findlay, 1976). Moffett et al. (1987) described 12 levator motor neurons, plus the common inhibitor, innervating the different heads of the levator muscle in *C. maenas*. Although we backfilled more neurons in *L. emarginata* than described by Moffett the locations of the somata within the ganglia were similar to those described for *C. maenas* (Fig 4.6; Fig 3 in Moffett et al., 1987). In *L. emarginata*, the posterior levator muscle splits into two different muscles in the third to fifth pereopods (Chapter 3), and the additional somata seen in *L. emarginata* could be associated differentially with these heads. This conjecture awaits further investigation.

## **Depressor Excitators**

The depressor motor neurons in *L. emarginata* resemble in number and location those described for the forward walking squat lobster (Fig 4.7; Fig 10 in Antonsen and Paul, 2000) and are more numerous than in the shore crab (Fig 7C in Bévengut et al., 1983). The large number of somata backfilled through the depressor nerve seems in agreement with the trend of increased number of somata accompanying increased number of muscle heads (compare *C. maenas* with *M. quadrispina*, Bévengut et al., 1983; Antonsen and Paul, 2000).

## **Promotor and Remotor Excitators**

The promotor and remotor excitators are located in the same ganglionic regions as described for other crustaceans (El Manira et al., 1991b; Bévengut et al., 1983). As in the case of the levator and the depressor (above), the promotor and remotor pools have more motor neurons in *L. emarginata* than *C. maenas* (Bévengut et al., 1983). The possibility exists that we inadvertently stained sensory cells along with motor neurons (described by Paul and Bruner, 1999; Paul and Wilson, 1994). Due to the radial arrangement of the legs, the last pair of pereopods lie directly caudal to the TG. During our experiments, backfilling the remotor nerves of the last pereopods also backfilled motor neurons innervating the abdomen of the crab. This was further confounded by the fact that the first pair of abdominal neuromeres of *L. emarginata* lie directly above the medial portion of the eighth thoracic neuromeres. The abdominal flexor inhibitor of the first abdominal neuromeres, described by Paul et al. (1985), often exited the TG in a single posterior nerve splitting only after traveling most of the length of the thorax. For the reasons stated above, we only reported the number of remotor somata from anterior neuromeres.

## **Non-Motor Cells**

Backfills of the nerve containing the flexor motor neurons or the levator motor neurons also stained at least two inter-ganglionic processes (IGPs). These processes extend to the

proximal end of the ganglion and there join a track containing other axons continuing in the rostral direction, including axons backfilled similarly in more posterior neuromeres. Some somata were filled as far anterior as the third thoracic neuromere. Faulkes and Paul (1997) reported similar processes in *M. quadrispina* and suggested that they might be secretory in nature. Previous work on secretory neurons in crustaceans revealed somata with similar locations to the ones we observed, but the inter-ganglionic processes derived from them do not match those of the cells we observed (Siwicki et al., 1985; Rossi-Durand, 1993; Antonsen and Paul, 2001).

## **Conclusions**

The neural anatomy of *L. emarginata* is similar to that described for a variety of other decapod crustaceans and resembles that of distantly related but forward walking anomurans and macrurans rather than the closely related and sideway walking brachyurans (Table 4.2).

**Table 4.2.** Comparison of leg motor neurons between *L. emarginata* and other crustaceans.

<i>Species</i>	<i>Carcinus maenas</i> <sup>1</sup>	<i>Libinia emarginata</i>	<i>Procambarus clarkii</i> <sup>2</sup>	<i>Munida quadrispina</i> <sup>3</sup>
walking preference	sideway	forward	forward	forward
infraorder	Brachyura	Brachyura	Astacidea	Galatheidæ
<b>Muscle</b>	Number of somata present			
Closer	2	2	2	2
Opener	1	1	1	1
Stretcher				
Extensor	2	2	2	1
Flexor	6	7	4	2
Acc. Flexor	1	1	1	1
Reductor	2	2	2	2
Levator	8	16	19	
Depressor	10	20	12	17
Promotor	7	17	19	
Remotor	7	11	13	
Inhibitors	3	3	3	3
Total estimate	49	82	78	

1) After Bévengut et al., 1983; Moffett et al., 1987; Parsons, 1982. 2) After El Manira et al., 1991a; El Manira et al., 1991b; Pearlstein et al. 1995. 3) After Faulkes and Paul, 1997; Antonsen and Paul 2000. Spaces signify numbers not reported.

Spider crabs belong to a group postulated to be among the first to undergo the process of becoming crab-like (Rice, 1983; Morrison et al., 2002) which makes them interesting from a phylogenetic perspective. Also, as a forward walking brachyuran, neuroethological analyses of their walking behavior promises to be especially informative of the organization and evolution of legged locomotion in crustaceans.

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## **CHAPTER 5**

### **INTERLIMB DIFFERENCES IN THE KINEMATICS AND MUSCULAR ACTIVATION DURING FORWARD WALKING IN THE SPIDER CRAB, *LIBINIA EMARGINATA***

## INTRODUCTION

A wide range of interesting behaviors and their amenability to experimental techniques have made crustaceans preferred organisms in neuroethology for many decades. The study of legged locomotion is no exception, and much was learned about the production of pedestrian locomotion from studies on crustaceans (Clarac, 1982; Macmillan, 1975; Paul, 2006). Although they can walk in any direction, crustaceans can be grouped into those that preferentially walk forward (crayfish, lobsters) and those that walk sideways (crabs). Comparisons between these two modalities of pedestrian locomotion may help us understand the requirements imposed by legged locomotion in general. Studies of evolutionarily related animals with different walking strategies may also shed light on the changes required in the nervous system and the periphery during the evolution of new behaviors.

Many of the studies of crustacean walking behavior were conducted on reduced preparations (Elson et al., 1992; Sillar et al., 1987) or on tethered animals walking on treadmills (Ayers and Davis, 1978; Clarac and Chasserat, 1979; Clarac, 1982; Cruse and Saavedra, 1996). Only a few studies focused on the performance of walking behavior in freely walking animals (Barnes, 1975; Hui, 1992; Macmillan, 1975; Martinez et al., 1998; Sleinis and Silvey, 1980). Still fewer studies looked at either the kinematics (Jamon and Clarac, 1997; Martinez et al., 1998) or the muscular activity of the legs while animals are freely behaving (Burrows and Hoyle, 1973; Clarac et al., 1987). Notable exceptions are the reports by Ayers and Clarac (1978) and by Atwood and Walcott (1965) that examined both limb kinematics and muscle activation in freely-behaving lobsters and crabs respectively.

The spider crab, *Libinia emarginata*, is a brachyuran that walks preferentially forward about 80% of the time (Schreiner, 2004). As a majoidean crab, *L. emarginata* belongs to a group of crabs thought to have first adopted the crab shape from lobster-like ancestors (Rice, 1983;

Morrison et al., 2002). Their phylogenetic position and walking preference make spider crabs interesting subjects for comparative studies on the evolution of sideways walking among brachyurans.

Spider crabs possess skeletal adaptations that reflect their walking preference. These include larger ranges of motion for joints articulating in the horizontal plane that are used in forward locomotion and a more radial arrangement of the limbs around the thorax than seen in most brachyurans (Vidal-Gadea et al., 2008). The musculature of the legs of *L. emarginata* has a larger number of proximal muscle bundles than that of sideways walking brachyurans, but is similar to forward walking lobsters (Chapter 3). A concomitant increase is seen in the number of motor neurons innervating this proximal musculature (Chapter 4). These motor neurons are similar in number and location to those of forward walking lobsters (Antonsen and Paul, 2000). Work on the depressor and levator musculature of the squat lobster led Antonsen and Paul (2000) to hypothesize the potential independent activation of the different bundles during locomotion.

The legs of sideways walking crabs can pull or push the animal depending on the direction of locomotion. Sideways walking is advantageous in reducing overlap between adjacent limbs and potentially permits larger ranges of motion in the direction of locomotion. Being able to walk in two directions equally well makes crabs fast unpredictable prey and predators, but also prevents the appearance of specializations for walking in one particular direction (see Ritzmann et al., 2004). Since spider crabs walk mostly forward, we hypothesized that their limbs would show differences in kinematics and muscular activation depending on their anterior or posterior locations.

The present study is part of a series of studies investigating the neuroethology of forward walking in spider crabs. We characterized the kinematic behavior of all the legs and the majority

of the joints of spider crabs freely walking both forward and sideways. We did not include the basischiopodite-meropodite (BM) joint in this study (see Table 5.2 for abbreviations). This joint rotates the long axis of the leg anteriorly and has the smallest range of motion of all the joints of the leg (Vidal-Gadea et al., 2008).

In order to determine if anterior and posterior legs exhibited differences in behavior, we focused on the third and fifth pereopods. The depressor musculature of spider crabs consists of multiple muscle bundles and is involved in posture and the power stroke of locomotion. Some of these bundles are entirely contained within the coxa joint, while others originate in the thorax (Chapter 3). We recorded simultaneously from different depressor bundles in legs three and five during free walking. The coxal and thoracic bundles are activated phasically, but often independently, during locomotion. Simultaneous kinematic data revealed that differences in depressor firing rates between the legs coincides with different ranges of motions for these two legs during forward walking.

**Table 5.1.** Abbreviations used in this study.

CB	Coxa-basipodite joint
COM	Center of mass
CP	Carpo-propodite joint
L1-L5	Left leg 1 through 5
MC	Merus-carpus joint
PD	Propo-dactylopodite joint
R1-R5	Right leg 1 through 5
TC	Thorax-coxopodite joint

## **MATERIALS AND METHODS**

### **Animals**

*Libinia emarginata* ranging in size from seven to ten cm in length were obtained from the Marine Resources Center of the Marine Biological Laboratories in Woods Hole, Massachusetts. Crabs were kept at 20°C in artificial seawater until used.

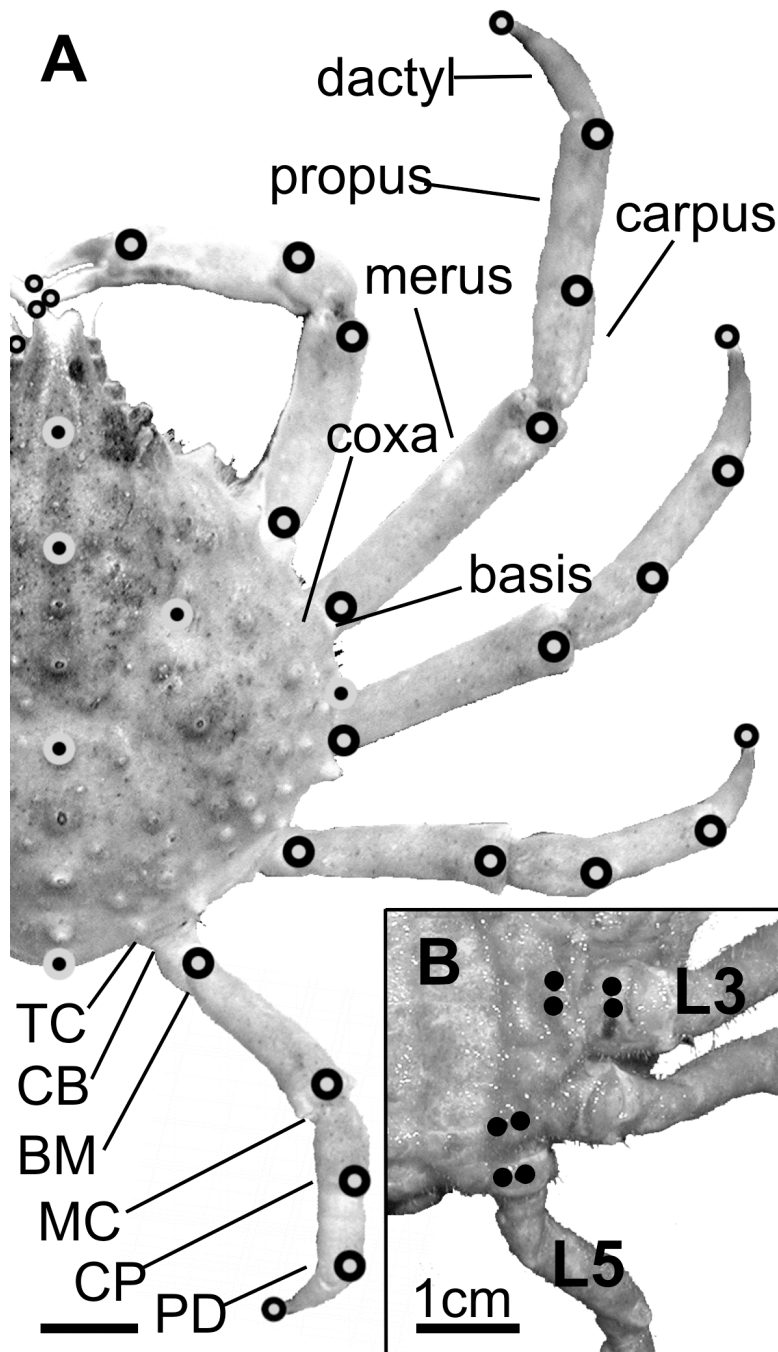
### **Kinematics**

The animals were cold-anesthetized for 30 minutes at 4°C. Points of interest were painted on each animal using a mix of 50% Krazy Glue® and black or white nail polish. Eight points were labeled on the carapace and four points each on the dorsal and ventral surfaces of each leg segment distal to the basischiopodite (Fig 5.1A).

The crabs were allowed to recover from the labeling procedure for 60 minutes in a 20-gallon aquarium containing a thin layer of black lava sand as substrate. Four cameras (three Canon GL-1 and one Canon ZR200) filming from different angles captured video of the animals performing their walking behaviors. The videos were subsequently imported into the Peak Motus Motion Measurement System (Ver 7, Peak Performance Technologies, Colorado), which was used to calculate three-dimensional trajectories for each of the points of interest. The same software was used to calculate joint angle changes. To compare anterior (leg 3) and posterior (leg 5) legs, we used six animals in seventeen trials for a total of 58 steps analyzed.

### **Electromyography**

The day after labeling, the animals were again cold-anesthetized prior to electrode implantation. Pairs of Teflon-coated silver wire electrodes 0.007 inches thick (A-M Systems, Washington) were inserted ventrally into the depressor musculature of the thorax and into the coxa of the right third and fifth legs (Fig 1B). We confirmed the successful placement of the electrodes by recording the depressor electrical activity and also by dissection after the experiment. The electrodes were fixed in place by gluing them to the carapace with a small piece of Kimwipe® soaked in Krazy Glue®. The free ends of the electrodes were attached to a male/female connector, which allowed us to unplug the animals between recording sessions.



**Figure 5.1.** Segments and joints of the spider crab, *Libinia emarginata*. A) Dorsal view of the right half of a spider crab showing the placement of the dots used during filming. The different segments composing the legs of the crab are labeled for the second leg, and the joints that articulate them are labeled for the fifth. B) Ventral view of the left third and fifth legs showing the location of electrode implants (black dots).

Using this procedure, the electrodes remained usable for several days if care was taken to protect their ends from salt water.

EMG recordings were amplified (A-M Systems 1700 amplifier with 100-5000Hz filtering). The data were processed by a computer with Datapac 2K2® software for storage and analysis. Datapac 2K2® was set to a 10,000Hz sampling rate for all recordings. To synchronize the video and EMG data, a square wave pulse was recorded with the EMG data and simultaneously used to drive a light-emitting diode in the field of view.

### **Data Analysis**

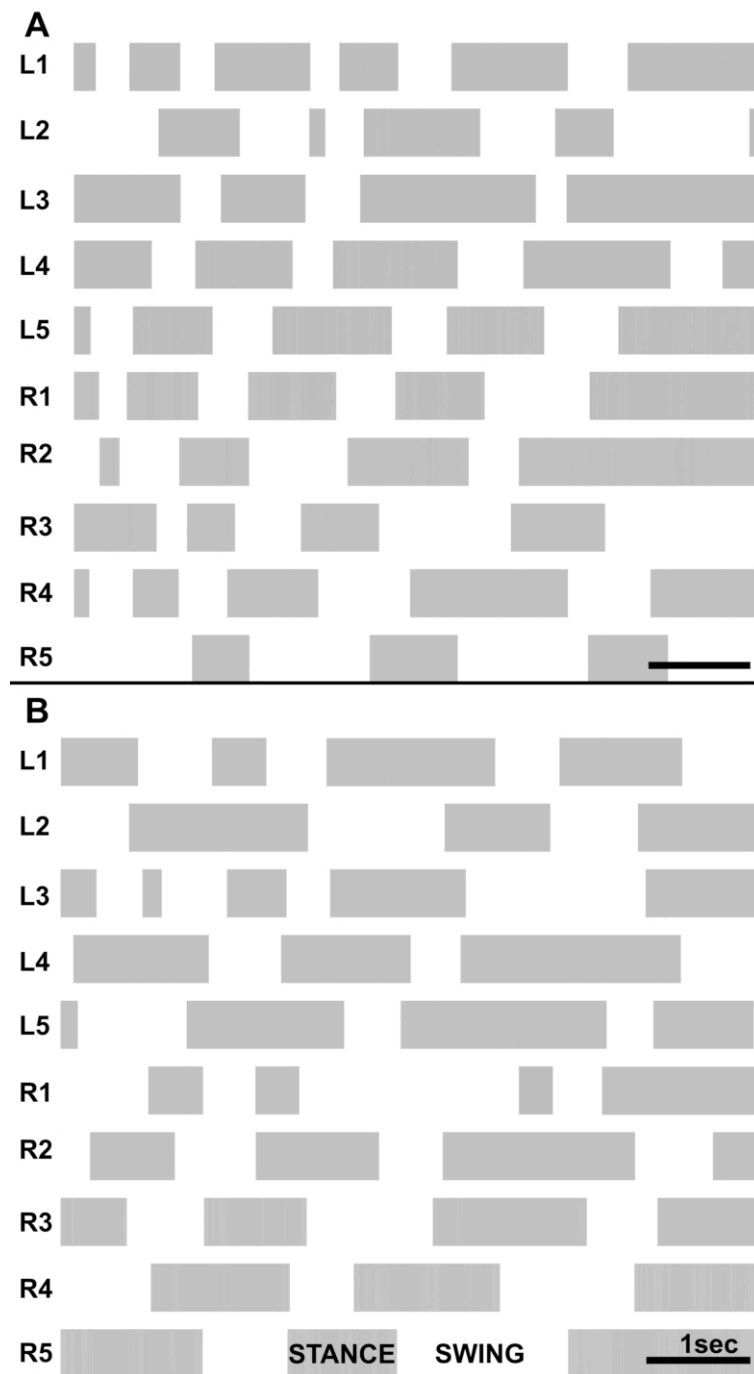
Ranges of motion and angular accelerations were plotted using Sigma Plot® and analyzed using Sigma Stat®. The software package R (Ihaka and Gentleman, 1996) was used to analyze inter-joint coordination by the method of cross-correlation functions (Jamon and Clarac, 1997). Briefly, time series of the angular trajectories of each joint were compared with the time series of the angular excursion of every other joint in the same leg. Confidence intervals were estimated as  $\pm 2/\sqrt{N}$ , where N is the number of observation pairs used to calculate the correlation.

## **RESULTS**

### **Leg Coordination During Locomotion**

To determine the stepping patterns (gaits) used by spider crabs, we measured the order in which the end of the stance phase occurred using the method of Sleinis and Silvey (1980) (Table 5.2). Spider crabs walk forward using most frequently an overlapping metachronal gait that travels in the anterior direction (Fig 5.2A, Table 5.2). As reported for the forward walking soldier crab, the fifth and second legs tended to step in phase (Sleinis and Silvey, 1980). Although anteriorly traveling metachronal gaits were previously reported for many insects (Bowerman, 1977; Burns, 1973; Delcomyn, 1971; Graham, 1972; Wilson, 1966) and other





**Figure 5.2.** Footfall patterns for a spider crab walking in the forward (**A**), and sideways (**B**) direction. Spider crabs use metachronal gaits during the performance of both behaviors. The legs are labeled from Left leg 1 on top (L1) to the right leg 5 (R5) on the bottom. Dark bars represent the stance part of the step during which the leg is in contact with the ground.

decapod crustaceans (Barnes, 1975; Macmillan, 1975) this gait is opposite to that reported for crayfish (Jamon and Clarac, 1995) and soldier crabs (Sleinis and Silvey, 1980). During sideways locomotion spider crabs also use anteriorly traveling metachronal (Fig 5.2B) and alternating tetrapod gaits (Schreiner, 2004).

**Table 5.2.** Comparison among the stepping patterns observed in spider crabs walking forward. The gaits correspond to 51 sequences from six crabs. Legs were labeled from anterior to posterior.

Gait					Count	%
4	3	5/2	1		10	19.6
4	3	2/5	1		8	15.7
4	3	1	2/5		8	15.7
4	1	3	5/2		7	13.7
4	1	3	2/5		4	7.8
4	3	1	5/2		4	7.8
4	3	5	1	2	3	5.9
4	1	2	3	5	1	2
4	3	2	1	5	1	2
<i>Total anterior direction</i>					<i>46</i>	<i>90.2</i>
4	5	1	2	3	2	3.9
4	5	3	2	1	1	2
4	1	5	2	3	1	2
4	1	5	3	2	1	2
<i>Total other</i>					<i>5</i>	<i>9.8</i>
Total					51	100

### **Individual Leg Contribution to Forward Locomotion**

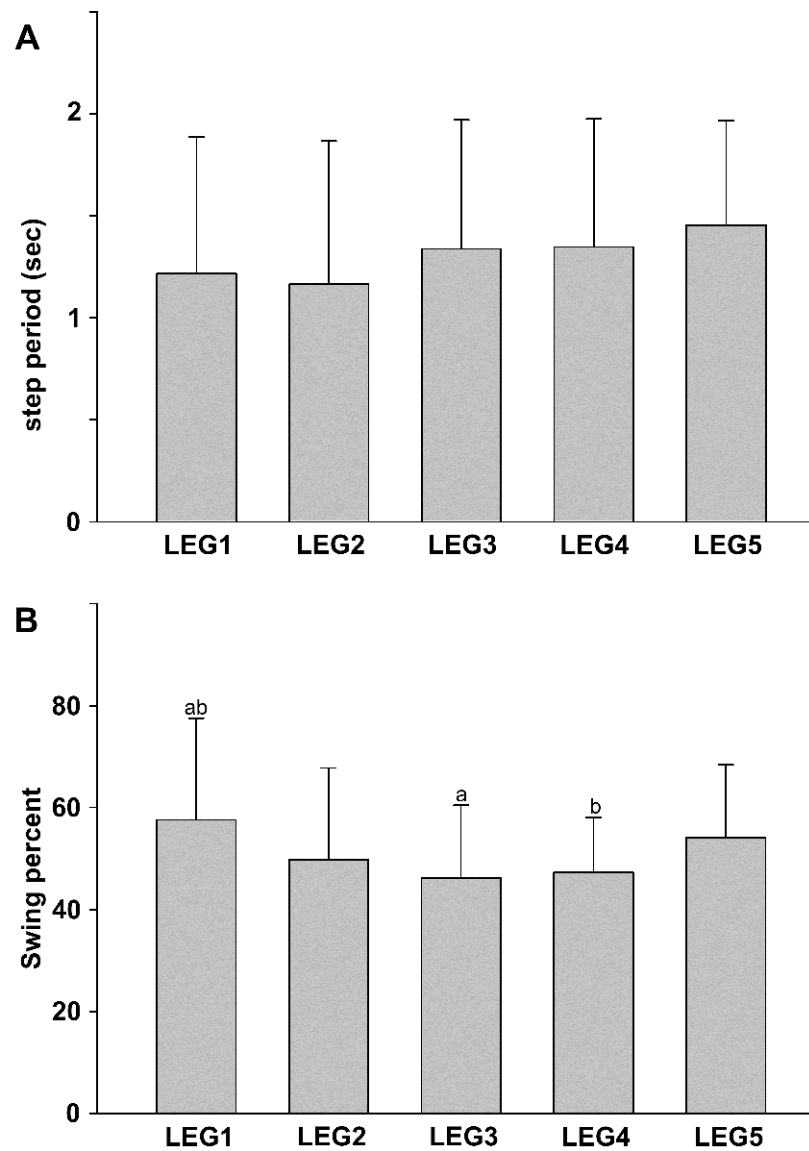
Although spider crab legs differ considerably in length and orientation, during forward walking the duration of the steps (step periods) is similar between the different legs (Fig 3A). Furthermore, most legs spend a similar percentage of each step in contact with the ground (Fig 5.3B). The only leg that showed a significant difference was the first leg, which had a longer swing than the third and fourth pereopods ( $p=0.012$  and  $0.009$ , respectively, Mann-Whitney t-test). These findings closely resemble those described for the crayfish (Jamon and Clarac, 1995).

### **Forward Walking**

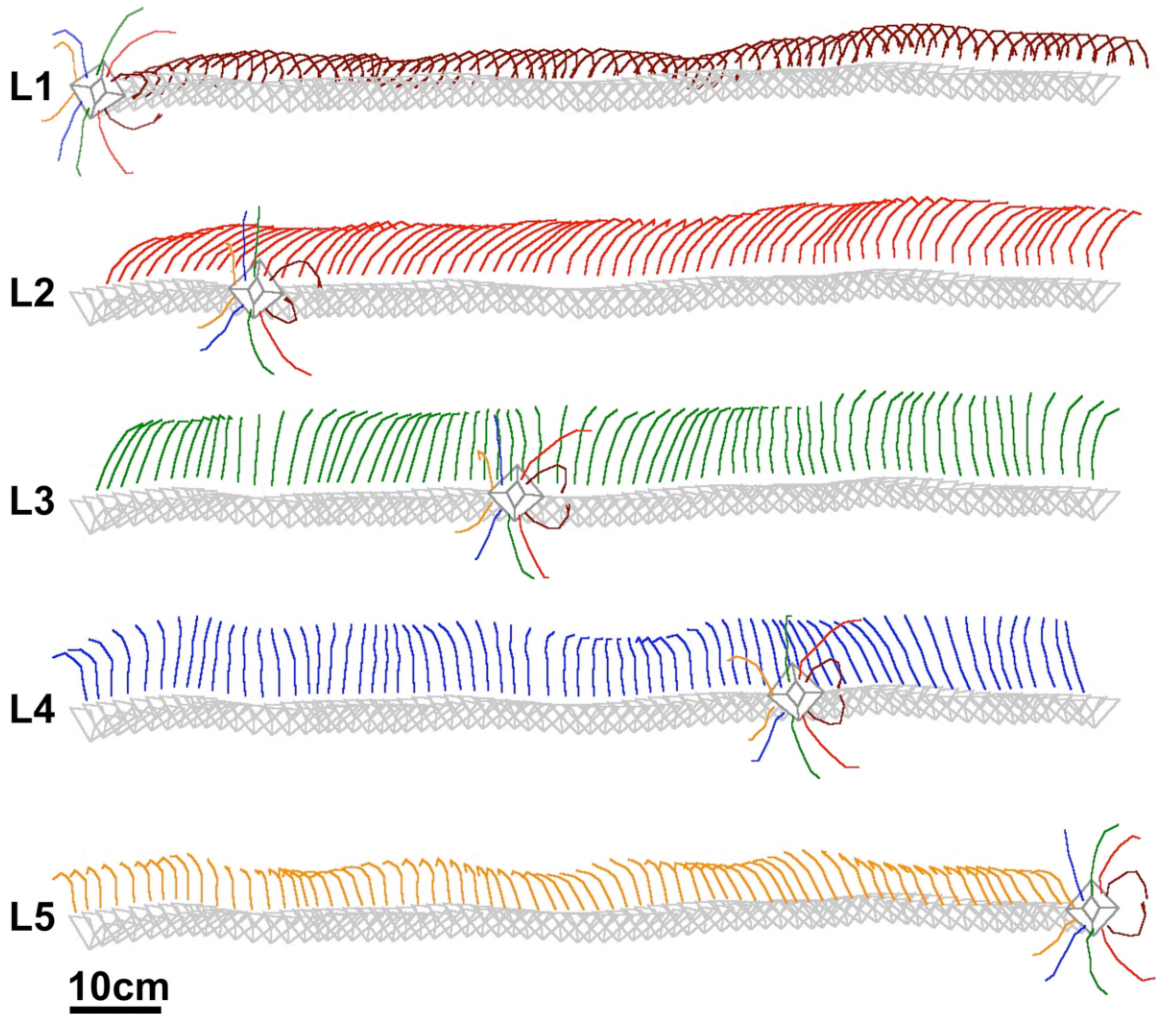
The legs of spider crabs perform different behaviors during forward walking (Fig 5.4). During forward walking, the last pereopod begins by levation at the CB joint and promotion at the TC joint. If the fifth pereopod begins the swing pointing in the posterior direction, parallel to the long axis of the body, then the step will typically set down before contacting the next anterior pereopod. If, on the other hand, the starting position of the pereopod is parallel to the anterior pereopods, perpendicular to the long axis of the body, then the end of promotion will begin upon contact with the next anterior leg. Under these latter conditions, the fifth pereopod will typically cease promotion and levation and begin bending at the CP joint (maximizing forward progression of the dactyl). Contact with the fourth pereopod seems also to trigger levation and promotion (following Hughes (1952) rule for timing of leg promotion) and thus the continuation of the locomotor wave in the anterior direction. These sensory cues occur when one leg comes into contact with another within its normal range of motion. If the normal range of motion is reached without the legs contacting each other then the anterior leg begins its return stroke without being contacted by its posterior counterpart. During the power stroke, remotion and depression of the TC and CB joints, respectively, are followed by extension of the MC, CP, and PD joints. The description above applies in most instances to legs four and five (posterior to the center of mass). The first and second legs, however, lie anterior to the center of mass and pull rather than push on the substrate by flexion of the MC and PD joints. Because the third leg lies perpendicular to the long axis of the body, it will either pull or push (or both) the substrate depending on its starting orientation (Fig 5.5).

### **Sideway Walking**

During sideway walking the legs of spider crabs perform different jobs depending on whether they are leading or trailing. In addition, legs anterior and posterior to the center of mass



**Figure 5.3.** Stepping periods and durations of swing for the different pereopods of *L. emarginata*. **A)** The average duration of a step does not differ significantly among the different pereopods of spider crabs. **B)** The swing phase of the steps does not vary greatly among different legs and is roughly 50% of the step period. Legs differing significantly from each other on the swing percent are labeled with the same letter.



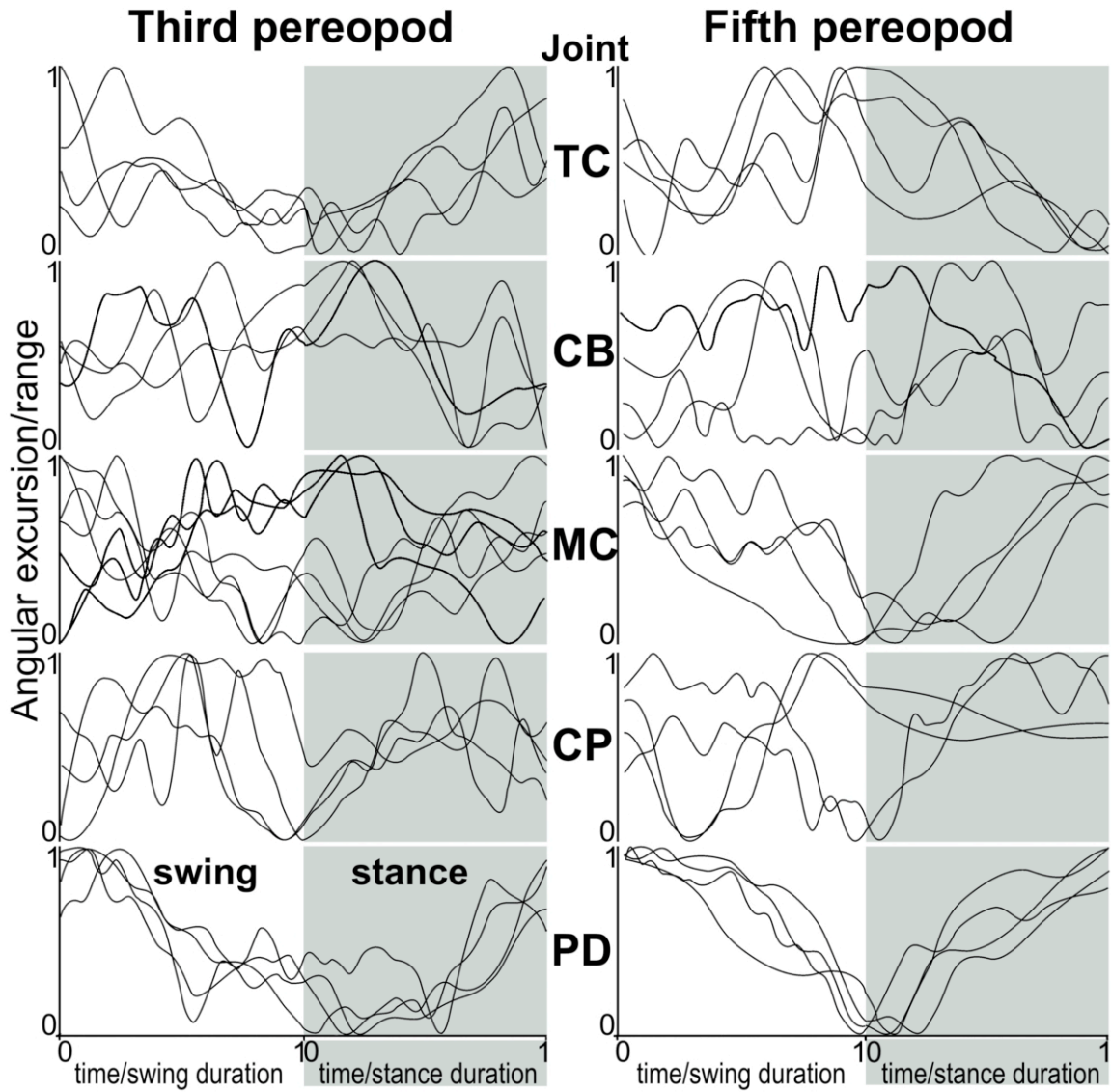
**Figure 5.4.** Cartoon representation of the sequence of motions undergone by the left legs of spider crabs during a forward walking bout shown in dorsal view. The stick figures representing the legs show the position of the four distal leg segments every 50 ms during three consecutive steps.

perform different behaviors. Trailing legs anterior to the center of mass have a swing phase consisting of flexion of the TC, CB, MC, CP, and PD joints. During the stance phase of the step, anterior trailing legs engage in the opposite motions (depression, remotion, extension, stretching, and opening respectively). Legs four and five are posterior to the COM and have a different pattern of activity. During the stance phase, the posterior trailing legs promote the TC and bend the CP joints, which has the effect of pushing the animal along a vector that is perpendicular to that of anterior legs and roughly 45 degrees to the direction of motion. When combined this produces propulsion in the direction of locomotion. If this partial antagonism between the action of anterior and posterior legs were not present, trailing leg activity would result in rotation of the animal in a clockwise if all legs remoted the TC joint or in a counterclockwise direction if all the legs promoted it.

During sideways walking, the leading legs of spider crabs also perform different behaviors depending on their position with respect to the COM. During the stance phase, the posterior legs promote and stretch the TC and CP joints, respectively, while the anterior legs remote and bend them.

### **Joint Trajectories**

During walking, the joint excursions of the legs of *L. emarginata* exhibit small oscillations (Fig 5.5). This is typically characteristic of limbs relying heavily on sensory feedback (Epstein and Graham, 1983). In addition to the variability observed within leg trajectories, the legs of spider crabs also show differences in joint excursions between anterior and posterior pereopods and differences depending on the starting position of the limb (Fig 5.5). The differences in the orientation of legs three and five with respect to the long axis of the body (Fig 5.1) create different trajectories of the TC and CB joints (Fig 5.5). Because the long axis of the third leg is oriented in the anterior direction the angle of TC decreases during the swing



**Figure 5.5.** Examples of joint excursion variability during forward walking in the third and fifth pereopods of *L. emarginata*. The trajectories were normalized for angular range, and for swing and stance durations to aid comparison. The joints are presented from proximal (top) to distal (bottom). The small oscillations observed in each joint were present in every animal and trial.

phase of the step and increases during the stance. The opposite is observed for the fifth leg, where the posterior orientation of the leg results in an increase of the TC angle during the swing and a decrease during the stance. Likewise, the angle of the CB joint for the third leg is unimodal as this leg maintains a dorsal heading throughout the step cycle. The fifth pereopod in contrast alternates between ventral and dorsal orientation throughout the step cycle resulting in a bimodal joint trajectory (Fig 5.5).

Because the third pereopod is perpendicular to the longitudinal axis of the body, the MC joint is sometimes used to pull the animal (e.g. if the leg started the stance pointing anteriorly) or push it (e.g. if stance began with the limb pointing posteriorly, Fig 5.5).

### **Ranges of Motion**

For comparative purposes, we measured simultaneously the joint angle changes in all joints of all of the limbs of a single individual (Fig 5.6). To examine more closely differences between anterior and posterior pereopods, we made more extensive observations of pereopods 3 and 5 from six animals during seventeen forward walking trials.

During forward walking, the ranges of motion for the different joints of the legs of spider crabs do not vary considerably. During sideway walking this trend remains with the exception of the fifth pereopod which generally has a larger range of motion than the more anterior pereopods (Fig 5.6).

The trailing legs have a reduced range of motion and variability when compared to the leading legs or to all legs during forward walking. As with the leading legs, the fifth pereopod has a larger range of motion compared to anterior legs.

### **Angular Accelerations**

The angular acceleration of the joints during forward and sideway locomotion reflected the findings for the ranges of motion (Fig 5.7). For all three types of walking behavior, the



acceleration of the TC joint was the smallest and showed the least variability. During forward walking, the CB joint also had small accelerations during the swing and the stance phase although both the values and their variability increased during sideway walking. The greatest joint accelerations occurred for joints with the largest and most variable ranges of motion, which included the PD, CP, and MC joints of the leading fifth pereopod during sideway walking.

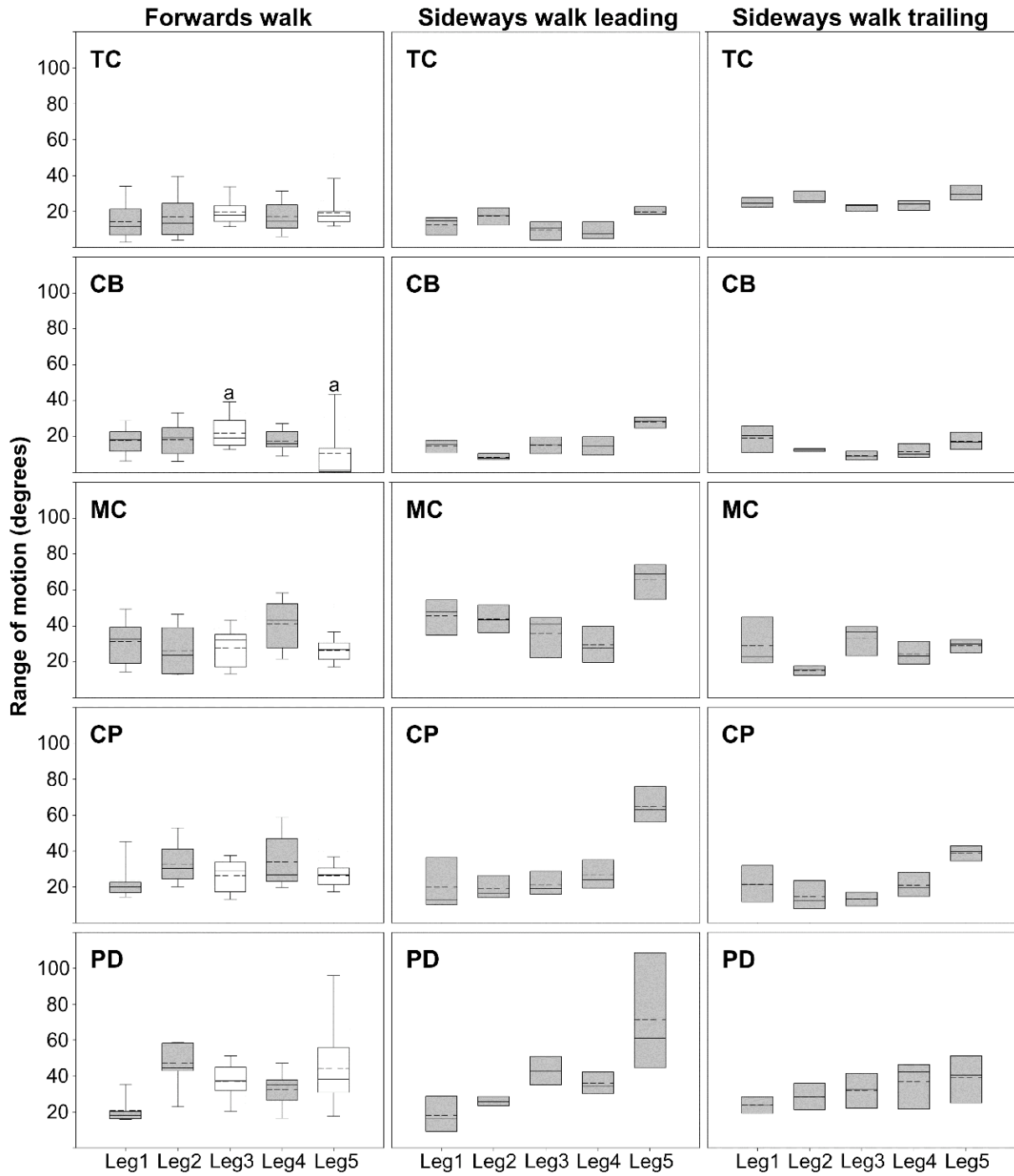
For most joints, the magnitude of the accelerations during the swing phase of the stance was matched by accelerations of comparable magnitudes and opposite sign in the stance phase.

### **Inter-Joint Coordination**

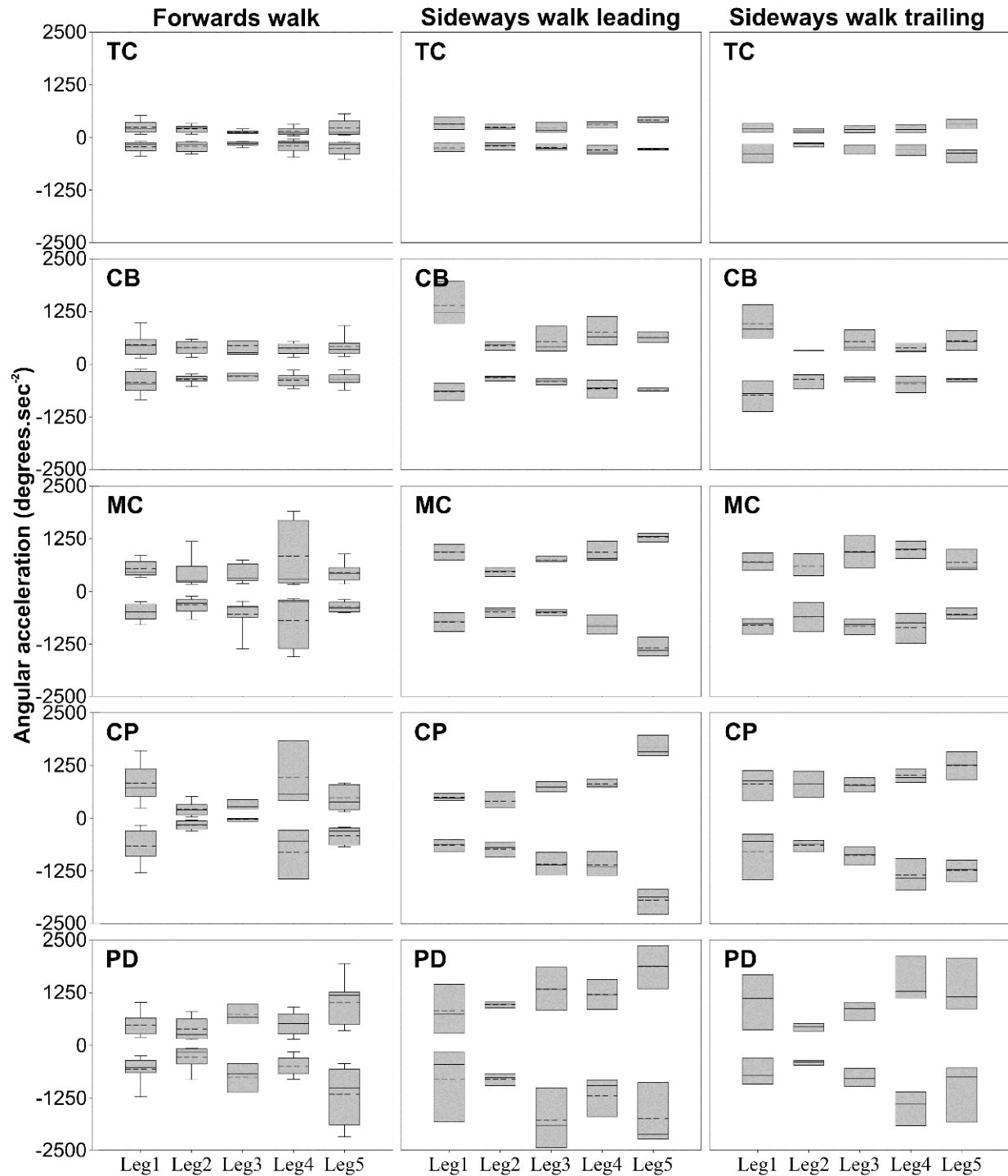
Cross-correlation functions allow comparisons between different joints and highlight the coordination, or lack thereof, between any two joints of interest (Jamon and Clarac, 1995). Comparison among the leg joints of the third and fifth pereopod revealed different patterns of cross-correlation (Fig 5.8). For example, the strongest correlation observed between any two joints in legs three and five was between MC and PD in leg five. Here, the correlation between joints MC and PD peaks around a time lag of zero indicating that both joints increase and decrease their angles at the same time. In general, the cross-correlation matrix (Fig 5.8) shows higher correlation among the joints of the fifth pereopod than among those of the third. Another consistent pattern is that joints that are distant to each other tend to have higher cross-correlation coefficients. Conversely, joints that are adjacent to each other (i.e. close to the diagonal in the matrix) tend to have low cross-correlation coefficients.

### **Depressor Bundle Activation During Walking**

Multiple muscle heads are responsible for articulation of the proximal joints (Chapter 3). We were interested in whether these muscles are ever activated independently during locomotion and if there are differences in activity between anterior and posterior legs. We chose to focus on the depressor musculature because it lies most ventral in the animal, is easily accessed, and



**Figure 5.6.** Box plots of the ranges of motion for the joints of the legs of *L. emarginata* during forward and sideways walking (both trailing and leading). The joints of the legs of *L. emarginata* are displayed from proximal (top) to distal (bottom). The boxes show the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles with the solid lines indicating the median and the dotted lines indicating the mean. Bars with the same letter are significantly different. The gray boxes were obtained from a single animal and represent an average of six steps per leg. The white boxes represent 16 trials from five animals (20 steps per leg).



**Figure 5.7.** Maximal angular acceleration of the joints of *L. emarginata* during forward and sideways (leading and trailing) walks. The joints of the legs of *L. emarginata* are displayed from proximal (top) to distal (bottom). The boxes show the 10<sup>th</sup>, 25<sup>th</sup>, 75<sup>th</sup>, and 90<sup>th</sup> percentiles with the solid lines indicating the median and the dotted lines indicating the mean. Positive accelerations correspond to the swing, and the negative accelerations to the stance phase of the step. The boxes were obtained from a single animal and represent an average of six steps per leg.

because the ranges of motion of the CB joint differed significantly between the third and fifth pereopods (Fig 5.6). Recordings from freely walking crabs performing forward walks were obtained from the coxal and thorax-bound heads of the depressor musculature of the third and fifth pereopods. This allowed us to compare the activity in different depressor bundles to each other, to their counterparts in another leg, and to the angular excursion of the CB joint they move (Fig 5.9). Although the different depressor bundles are both active during forward locomotion, their activities are not identical, demonstrating their independent activation at least part of the time (Fig 5.9, 5.10).

The patterns of activity of different depressor bundles also suggested a separation in their roles. While the depressors of the third pereopod were activated nearly simultaneously every time (Fig 5.11A), those of the fifth varied considerably in the timing of their activation (Fig 5.11B).

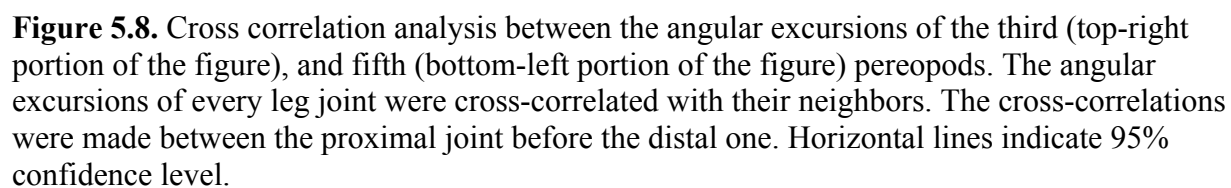
### **Depressor Firing Frequency Versus Range of Motion**

The larger range of motion observed for CB in leg three than in leg five ( $21.5 \pm 9.3^\circ$  versus  $10.3 \pm 16.3^\circ$  respectively,  $p=0.014$ , Holm-Sidak ANOVA) was accompanied by a higher depressor firing frequency in leg three ( $40.2 \pm 15.5\text{Hz}$  versus  $22.3 \pm 2\text{Hz}$  respectively,  $p<0.001$  Holm-Sidak ANOVA).

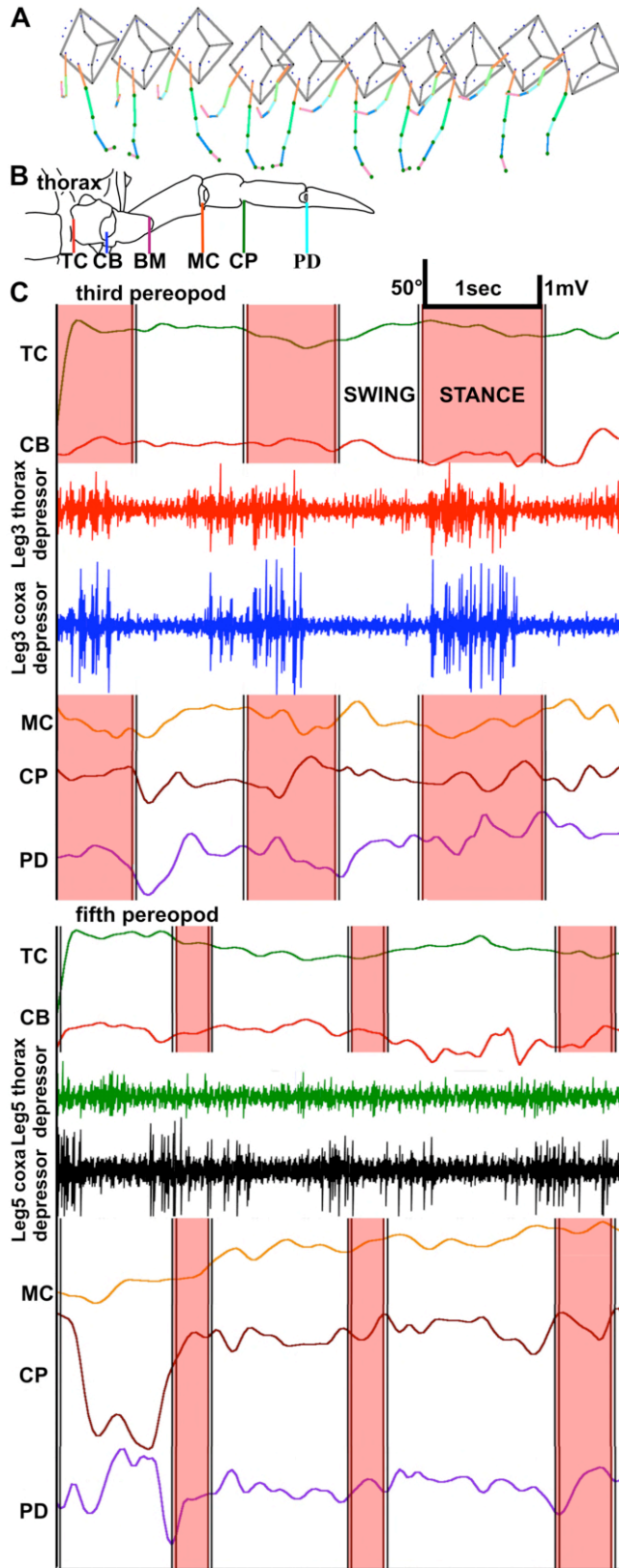
## **DISCUSSION**

### **Leg Coordination During Locomotion**

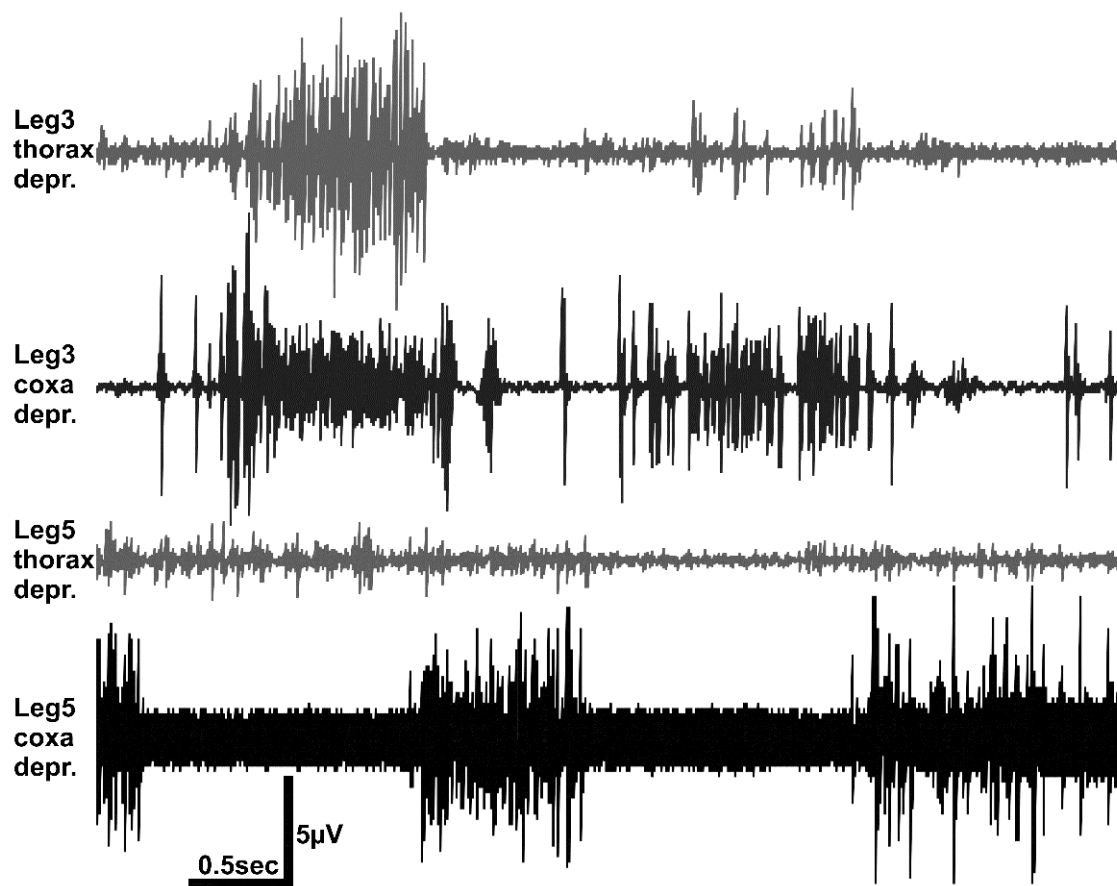
We chose to study locomotion in *Libinia emarginata* because it is a brachyuran crab that, unlike other brachyurans, walks forward most of the time. Spider crabs use anteriorly traveling metachronal waves to walk forward and sideways (Fig 5.2). Previous work on forward walking soldier crabs (Sleinis and Silvey, 1980), crayfish (Jamon and Clarac, 1995) and lobsters (Macmillan, 1975) showed these decapods use posteriorly traveling metachronal gaits to walk



forward. Furthermore, comparisons by Clarac (1982) of the stepping patterns described for several decapods showed that the 2534 stepping pattern was the most common among the species described regardless of walking preference. Curiously, this is the only stepping pattern we did not observe in spider crabs (Table 5.2). This departure from the pattern demonstrated by other species could not be explained by the observation that, as in soldier crabs, legs five and two tend to act in phase thus confounding the 2534 and 5234 gaits. We observed the fourth leg step following the fifth and preceding the third in nearly all events suggesting a true anteriorly directed metachronal gait. The COM of spider crabs is more posterior than in crayfish and shore crabs (Vidal-Gadea et al., 2008), which results in more legs laying posterior to the COM in spider crabs thus pushing (rather than pulling) the COM during forward-locomotion. It is possible that an anterior traveling metachronal wave would be favored since this would give precedence to the legs providing the propulsive force. Furthermore, the radial arrangement of the legs around the thorax of *L. emarginata* (Vidal-Gadea et al., 2008) results in the fifth pereopods having the widest available range of motion in the horizontal plane (at the TC joint). In addition, the fifth pereopods at rest are oriented posteriorly; therefore, forward propulsion by anterior legs could result in dragging of the posterior pereopods. During our experiments, we only observed posteriorly traveling gaits on the rare occasions when crabs walked backward. During sideway locomotion, spider crabs also use an anteriorly traveling metachronal gait. With the exception of fiddler crabs (Barnes, 1975), the use of an anteriorly traveling metachronal gait for sideway locomotion is different from the alternating tripod and tetrapod described for other brachyurans walking sideways (Burrows and Hoyle, 1973; Schreiner, 2004). The use of a single gait for accomplishing such dissimilar behaviors may be related to spider crabs' basal status as brachyurans and could offer insight into how the transition between forward and sideway walking behavior took place in crab evolution.

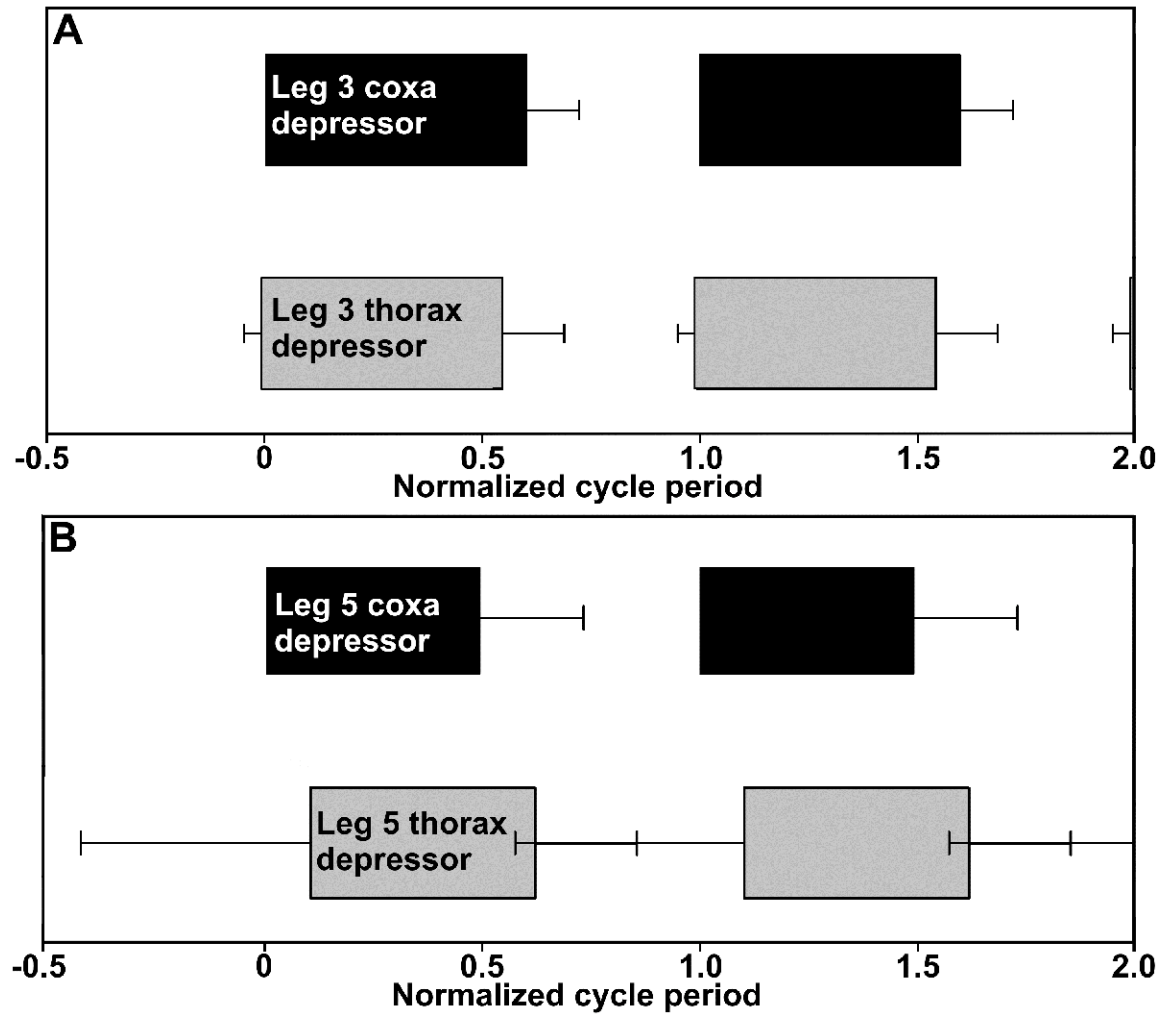


**Figure 5.9.** Forward walking behavior of *Libinia emarginata* showing the angular excursions of the joints of the third and fifth legs, and the activity of the thorax and coxa bound depressor bundles. **A)** Stick figure representation of the change in position over time of the points labeled in the legs and thorax during a forward walk. The figures show schematically the position of the limbs every 470 ms over the course of three steps. **B)** Joints of the legs of *L. emarginata*. **C)** Changes in joint angle for each joint of the third (top), and fifth (bottom) legs during these steps. The activity in the coxa and thorax-bound depressor bundles is shown beneath the angular trajectory of the joint they move (CB). The joints are presented from proximal (top) to distal (bottom). The open blocks denote the swing phase of a step (no ground contact), while the pink blocks denote the stance phase (ground contact).



**Figure 5.10.** Depressor muscle activities in the third and fifth pereopods. Physiological recordings from the thorax and coxa-bound depressor bundles showing independent activation during a forward walking bout.





**Figure 5.11.** Averaged depressor bundle activity for legs three (**A**), and five (**B**) during forward walking. Black bars show the duration of the bursts in coxa-bound depressors, while the gray bars refer to the thorax-bound depressors. The left lines indicate the standard deviations of the onset, while the bars show the mean duration of the bursts with their standard deviations (right lines).

## **Individual Leg Contribution to Forward Locomotion**

The finding that all legs have the same step cycle and percent swing indicates that leg one is actively recruited during locomotion and is not just passively making occasional contact with the ground. Most descriptions of crustacean pedestrian locomotion do not include the first pair of pereopods, although Hui showed that a shore crab (*Pachygrapsus crassipes*) would occasionally use its chelipeds when walking on land (Hui, 1992). The only other crab shown to regularly use all ten legs during locomotion is the only other forward walking crab previously studied, the soldier crab *Mictyris platycheles* (Sleinis and Silvey, 1980). Spider crabs and soldier crabs are distantly related brachyurans as *L. emarginata* is a heterotreme and *M. platycheles* is a thoracotreme. Both species use all ten legs to walk forward. Crabs (Burrows and Hoyle, 1973) and cockroaches (Full and Tu, 1991) both show a tendency to use fewer legs at increasing speeds. Spider crabs walk slowly and thus make use of all ten pereopods. At the slow speeds that spider crabs walk, the recruitment of the first pair of pereopods (Fig 5.3) is in agreement with the trend described for other animals. As previously found for soldier crabs (Sleinis and Silvey, 1980) and for crayfish (Jamon and Clarac, 1995), all the legs of spider crabs had similar step periods and duty cycles, but this does not mean that every leg contributes equally to forward-propulsion. As in crayfish (Klärner and Barnes, 1986; Jamon and Clarac, 1995), each leg of spider crabs contribute in a specific way to forward locomotion.

## **Typical Walk**

### **Forward Locomotion**

One of the hypotheses I set out to test was that during forward walking limbs anterior to the COM show differences in kinematics when compared to limbs posterior to the COM. The third and fifth legs, for example, differ in the way they use the MC joint. Leg five uses the MC joint to push the ground during the stance phase. Leg three might use it similarly if the leg is

pointing back or use MC to pull the ground if the leg is pointed anteriorly (Figs 4,5). These differences in use of the musculature between anterior and posterior legs open the possibility for a divergence in muscular adaptations between anterior and posterior limbs.

### **Sideway Locomotion**

During sideway locomotion the legs of spider crabs behave differently from those of other sideway walking brachyurans (Martinez et al., 1998). Unlike the parallel orientation and action of the legs in other brachyurans, the anterior and posterior trailing legs of spider crabs push the crab along directions perpendicular to each other. However, the motion resulting from the sum of both vectors lies along the direction of locomotion. While leading legs use their PD and MC joints to pull the animal, spider crabs do not make the extensive use of the MC joint reported for other brachyurans (Burrows and Hoyle, 1973).

### **Range of Motion**

Although some variability is present between the use of different joints during locomotion in spider crabs, forward locomotion seems to be the product of multiple joints acting together and not the result of a main participant (Fig 5.6).

When I compared the range of motion of legs anterior to the COM (leg 3) to that posterior to it (leg 5), I found a significant difference in the range of motion only at the coxa-basipodite joint (Fig 5.6).

### **Joint Trajectories**

The trajectories of the joints during forward walking are highly variable and depend on the starting position of the leg as well as on the position of the neighboring ipsilateral limbs (Fig 5.5). Martinez et al. (1998) predicted and found that animals had greater kinematic variability walking underwater than on land. The different forces acting on animals walking in water, in particular the reduced effect of gravity because of buoyancy, permit greater flexibility in joint

trajectories when compared to pedestrian locomotion on land (Martinez, 1996). I observed slow oscillations in the trajectory of the limbs in every type of behavior. These oscillations are present for all joints and limbs and resemble those present in arthropods that rely heavily on sensory input during locomotion (Dürr, 2001; Epstein and Graham, 1983).

### **Inter-Joint Coordination**

Cross-correlation analysis was successfully used in studies of multi-joint coordination (Jamon and Clarac, 1997; Domenici et al., 1998; Cheron et al., 1998). One of the advantages of this method is that it allows the detection of patterns of coordination. The ranges of the coefficients found in this study are consistent with those described previously for crayfish (Domenici et al., 1998). The cross-correlations seen for the joints of the third leg suggest that TC has the highest correlation with the rest of the joints followed by CB (Fig 5.8). Jamon and Clarac (1997) suggested TC as potentially having a higher rank in the leg joint coordination hierarchy in the crayfish. We found that distant joints showed higher correlation coefficients than adjacent ones. Previous work on *Astacus leptodactylus* showed no correlation between the distal and proximal joints during forward locomotion (Barnes, 1977).

The fifth pereopod showed higher cross-correlation coefficients than leg three (Fig 5.8). The stronger coupling between the joints of the fifth leg suggests that there may be something important about their relative timing. This hypothesis is supported by observation of the legs in the walking animal. The third pereopods usually have the beginning and end of their steps triggered by contact with adjacent legs. This is rarely the case for the fifth pereopod, where only occasionally is the end of the swing phase triggered by contact with the fourth pereopod. More often, the fifth pereopod completes its step cycle without contacting its neighbor, and therefore may be more representative of the central output to the legs. Because leg three is between two other limbs, the central output to this limb could frequently be superseded by sensory feedback

from its interaction with its neighbors. The lower cross-correlation coefficients seen in the third limb could then result from reflex loops overriding the central output.

In their study of the fourth limb of *P. clarkii*, Jamon and Clarac (1997) noted legs finished protracting before landing and suggested that this was in preparation to push during the power stroke. We also observed TC ending its protraction before the end of the swing phase. In spider crabs, however, the end of TC promotion does not signal the end of protraction. The distal joints of *L. emarginata* continue protraction after TC is completed (see TC versus CP comparison in figure 5.8). Similarly, the strongest correlation between any joints was found between MC and PD in the fifth pereopods. Both of these joints, like TC and CP, articulate in the same plane. The lag between these joints showed that proximal joints reached their maximal angular amplitude before the distal joints (Fig 5.8). The lag between the peak in the proximal and distal joints suggest that they may be timed to maximize displacement. Often times a leg ceased promotion after it contacted an anterior neighbor. This, however, did not cease the motion at the CP joint which continued bending and placed the dactyl proximal and anterior to that of its anterior counterpart. In slowly walking animals like spider crabs, heavy reliance on sensory information and maximizing the anterior placement of the dactyl might be a strategy that reduces the energy spent during forward walking.

### **Depressor Bundles Activation During Walking in Different Legs**

The different depressor bundles of *L. emarginata* showed independent activation during walking behavior (Figs 5.9, 5.10, 5.11). Previously, Antonsen and Paul (2000) hypothesized the differential activation of the various proximal bundles comprising the proximal musculature of crabs. Although we observed the depressor bundles of *L. emarginata* to be activated in phase during walking, their activity was not identical.

### **Depressor Bundles Activation for Different Legs**

Although the duration of the depressor bursts during forward walking was similar for legs five and three, the intra-burst firing frequency was greater for leg three depressors. These firing frequencies are in the lower range of those reported in the literature for faster walking brachyurans (Clarac et al., 1987) but are similar to those from slowly walking macrurans (Ayers and Clarac, 1978). In addition, depressor bundle bursting in the third leg was more closely coupled than in leg five where the thorax bundle had greater onset variability (Fig 5.11).

These findings suggest that not only are the different depressor bundles activated independently, but they might be used differently in different legs. Further studies on the differential use of proximal muscles could reveal the extent to which this musculature is employed differentially for different behaviors.

### **Conclusions**

The finding that distal joints have positive lags to proximal joints moving in the same plane suggests that distal joints add to the displacement produced by the proximal joints in a context-dependent way. For example, bending of the CP joint in leg three increases anterior dactyl placement only if the axis of the leg is pointed sideways or posterior. If the leg is pointed forward the same motion reduces anterior dactyl placement. This last point argues the need to include distal joints in models seeking to describe forward walking in crustaceans if a full understanding of this behavior is sought.

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**CHAPTER 6**  
**DISCUSSION**

## INTRODUCTION

The performance of adaptive behavior requires the coordinated effort of many of the systems comprising an organism. From the central patterns generated by the nervous system to their interaction with the muscular, skeletal and sensory systems, behavior needs to be finely coordinated with the states of each of these systems and the environment if it is to be successful.

The field of neuroethology uses a multidisciplinary approach to the study of behavior (Hoyle, 1984), which requires investigators to be open to techniques and approaches as different as the components that produce the behaviors under study. As the study of pedestrian locomotion advances, it becomes important to find organisms that can help us bridge the gaps in our knowledge of distantly related, but well-studied species.

The rhythmicity and complexity of pedestrian locomotion make this an interesting and yet approachable subject for neuroethology. Many animals face similar challenges in order to perform this behavior, which allows us to compare approaches and findings from dissimilar organisms. In order to fully understand this or any other behavior, it is necessary to study the interactions among the different systems performing this task. These studies can include skeletal, muscular, and neural anatomy and physiology, as well as kinematics and biomechanics.

Many reasons make *L. emarginata* useful subjects for neuroethology. Spider crabs are majoid brachyurans (Samouelle, 1819). As such, they belong to the group thought to have first undergone the process of carcinification (becoming crab-like)(Rice, 1983; Morrison et al., 2002). Although spider crabs are brachyurans, they walk forward rather than sideways most of the time (Schreiner, 2004). This makes them an interesting potential link between the sideway walking brachyurans and the forward walking macrurans. Finally, the thoracic ganglia of spider crabs is unobstructed by the leg musculature making them amenable to *in vivo* physiological recordings (Chapter 4).

In the previous chapters, I described some of the ways in which the structure and function of the skeletal, muscular and neural systems reflect the walking preference of spider crabs. In the following paragraphs, I shall discuss some of the insights into the production of legged locomotion obtained from studying spider crabs.

### **Contributions of this Work to Neuroethology of Locomotion**

#### **Skeletal Adaptations for Forward Walking**

In Chapter 2, I described how animals tend to be elongated in the preferred direction of locomotion. The ranges of motion for joints articulating in the forward direction are also larger for spider crabs than for related species that walk sideways (Fig 2.5). This trend is also present for the lengths of the leg segments where segments housing muscles moving the aforementioned joints are similarly longer (Fig 2.6). The legs of spider crabs are arranged radially around the thorax. During normal stance, the dactyls lie on an ellipsoid that has its long axis oriented in the direction of locomotion (Fig 2.7). These findings hint at the potential importance of minimizing energy expenditure during locomotion as well as stance. Crabs, like other aquatic animals, are always exposed to forces such as drag and acceleration reaction that need to be countered (Martinez, 1996). Skeletal adaptations that minimize the energy spent during locomotion or stance, such as being streamlined to reduce drag, are likely to be selected for as they free up part of this energy for other important tasks such as finding food or mates.

#### **Muscular Anatomy of the Legs of Spider Crabs**

Although the muscular system is important in the production of locomotion the walking musculature was completely described for only a few crustacean species (Cochran, 1935; Hessler, 1982). In Chapter 3, I described the entire complement of muscles moving the joints of each leg in spider crabs. I showed that the proximal musculature of spider crabs resembles that of other forward walking crustaceans (Antonsen and Paul, 2000) in having more muscle bundles

than are found in sideways walking crabs. The distal musculature, which is usually more conserved, also showed similarities with distantly related forward walking species. Additional flexor bundles including an intersegmental bundle similar to that seen in the isopod *Janirallata* (Hessler, 1982) are also seen in spider crabs. Differences in musculature between the anterior and posterior pereopods are likely due to the differences in tasks these limbs must perform. Since anterior legs always lead the center of mass, they must pull on the substrate, while the posterior limbs lie behind the COM and must push the substrate to achieve forward propulsion.

### **Neural Anatomy of the Legs of Spider Crabs**

The small number of motor neurons innervating the leg musculature of crustaceans was studied for many species. Few species, however, have their entire complement of leg motor neurons described (see Faulkes and Paul, 1997; Antonsen and Paul, 2000). In Chapter 4, I provide a description of all the motor neurons innervating the legs of the spider crab. Mirroring my findings in the leg musculature, I saw a larger complement of motor neurons innervating the proximal musculature when compared with related sideways walking species (Table 4.2). The numbers and locations of motor neurons are, in fact, similar to those in the forward walking squat lobster (Antonsen and Paul, 2000). Recent work on the depressor motor neurons (innervating one of the proximal muscles) of the crayfish showed that these motor neurons are subdivided into seven functional groups (Hill and Cattaert, 2008). In some cases, larger number of muscle bundles and motor neurons might reflect the presence of additional muscles not present in related species with alternative walking preferences.

### **Kinematics of Forward Walking**

Spider crabs walk forward using anterior traveling partial metachronal waves (Fig 5.2). In Chapter 5, I showed that the anterior and posterior limbs of freely walking animals vary in the range of motion of their joints as well as in the degree of coordination between them (Fig 5.8).

This is understandable in light of the radial arrangement of the limbs around the thorax, as forward propulsion will require a different set of motions for limbs position at different angles around the COM.

For the two limbs that I studied in detail (legs three and five), the cross-correlations among the joints of a leg were larger for joints that were far apart, which indicates that widely separated joints are more tightly coordinated than are joints close together. In addition, distal joints exhibited positive lags when compared with proximal joints articulating in the same plane, indicating a proximal to distal progression of motion. A potential reason for this lag is that distal joints are being used to maximize the length of the stride. Because the legs of spider crabs are radially arranged, maximizing the anterior placement of the dactyl will call for different CP excursions depending on the final position of the TC joint. Looking at two extreme examples might help illustrate this point. If promotion of TC ends with the merus perpendicular to the long axis of the body, bending at the CP joint would maximize the anterior placement of the dactyl. If, on the other hand, promotion of TC ends with the merus parallel to the long axis of the body, as is the case for anterior legs, then bending at the CP joint would actually decrease the anterior placement of the dactyl. By coordinating the action of these two distal joints, spider crabs could insure that during each step the dactyl would be placed as far anteriorly as possible. This may also occur in crayfish, as Jamon and Clarac (1995) found a similar lag between the end of proximal joint promotion, and the end of the swing phase in the fourth leg.

### **Differential Activation of Proximal Muscle Bundles**

The presence of multiple proximal muscle bundles and large motor neuron pools innervating them was described for many crustaceans (Cochran, 1935; Bévengut et al., 1983; Antonsen and Paul, 2000). At least some of these bundles can be independently activated during the performance of certain behaviors, such as limb autotomy (Findlay and McVean, 1977). In

Chapter 5, I showed that some of the depressor bundles are activated independently during walking (Fig 5.9, 10, 11). The proximal musculature of decapod crustaceans has a greater volume available and more attachment sites in the thorax than those for the distal musculature in the leg. It is perhaps not surprising to find a larger number of motor neurons innervating this musculature, which creates the potential for a separation of labor among some of the different muscle bundles acting on a particular joint.

### **The Big Picture**

The previous chapters showed that the performance of forward walking by spider crabs is accompanied by adaptations at every level of the structure of the walking machinery. The skeletal, muscular and neural systems responsible for producing this behavior all have modifications that tune them to the particulars of their task.

### **From Forward Walking Lobster to Sideway Walking Crab**

Sideway walking crabs are unique in the animal kingdom in that they walk preferentially in a direction that is perpendicular to the longitudinal axis of their body. No successful explanation was offered for the reason behind this remarkable trait. One thing we do know, however, is that the crab form proved to be so successful that it independently (and irreversibly) arose five times from lobster-like ancestors (Morrison et al., 2002). Some of the lessons learned from spider crabs can provide us with clues about the emergence of sideway walking among brachyurans.

### **Evolution of the Crab Form**

Early decapods are thought to have been lobster-like in shape (Schram et al., 1978). The fusion of somites into a thorax and a tailfan are basal developments in decapod evolution (Paul et al., 1985; Schram et al., 1978), which allowed the evolution of tailflipping behavior as a means to escape predation. Tailflipping, however, places certain limitations on the organism. The

abdominal musculature must be massive and quick in order to generate the necessary power during performance. In addition, the abdominal segments must have reduced scleritization of their ventral surface in order to allow the fast bending of its segments during behavior. In order for tailflips to be effective, the center of mass of the animal must be close to the articulating point between the abdomen and the thorax. When the COM is away from this point, the effectiveness of tailflipping may be compromised. The need to keep the COM close to the center of the animal limits the maximum size of the chelipeds which are used for defense and attack. As decapods grew larger, not only was the abdominal musculature unable to produce the larger forces required to propel larger animals away from harm, but it was also a liability as it offered the least skeletal protection against predators. An example of this potential transition can be found in lobsters, where the small juveniles have been shown to tailflip in response to a threat, but later as adults switch to meral spread in response to the same stimulus (Lang et al., 1977).

Larger animals unable to escape by tailflipping might have resorted to hiding the vulnerable abdominal arthroal membrane by folding their abdomen under the thorax, as is seen in the squat lobster (Antonsen and Paul, 2000). Once tailflipping ceased to be an effective defense, the location of the COM was free to change without compromising survival of the animals. The change in location of the COM allowed the chelipeds to become larger and replace the abdomen in defense, which has the additional value of being just as useful in attacking prey as in defending against predators. It should be noted that many extant macrurans, such as lobsters and crayfish, possess large chelipeds which they use in defense in lieu of tailflipping when fully developed (Lang et al., 1977). The allometric growth of the chelipeds, however, occurs late in development as the animals become sexually reproductive (Laufer et al., 2002) and need to engage in dominance-establishing confrontations (Schroeder and Huber, 2001). For such



animals, the presence of a large abdomen actually allows the chelipeds to become larger since the tail acts as a counterbalance to the weight of their large claws.

For animals that underwent abdominal reduction the resulting decrease in body elongation produced by tucking and reduction of the abdomen became an exaptation for sideways walking, as an animal could now experience the same drag walking in any direction (Martinez, 1996). Furthermore, the perpendicular arrangement of leg joints meant that sideways walking did not require the profound changes to the skeletal structure as those described in vertebrates (Hutchinson and Gatesy, 2000), but merely the use of alternative joints (Chapter 2).

### **The Evolution of Sideway Walking**

For animals free to walk in any direction, sideways walking might be selected for as this direction offers the least amount of overlap between the legs and allows for greater ranges of motion, horizontal displacement and speed during locomotion (Burrows and Hoyle, 1973). Sideways walking has the added benefit of allowing animals to walk equally well in two opposite directions, a skill not seen in other organisms. An example of changes in walking speed following reassignment of the joints used in locomotion is seen in vertebrates. The sprawled limb arrangement exemplified today by crocodilians limits the speed at which these animals run. During the evolution of both birds and mammals, the hip joints underwent a 90° rotation. This rotation caused joints previously articulating in a plane perpendicular to the direction of locomotion to now articulate in a plane parallel to this direction (Hutchinson and Gatesy, 2000). Because the joints of the legs of crabs articulate in planes perpendicular to each other, using the joints that articulate sideways rather than forward could be comparable to vertebrates rotating their legs 90°.

The phylogenetic position of spider crabs, their similarities to macrurans in walking structure and function all point to forward walking in *L. emarginata* as an ancestral trait.

Furthermore, sideways walking in spider crabs uses the same metachronal gait used in forward walking. The kinematics of sideways walking in spider crabs is unlike that of sideways walking brachyurans and is suggestive of a transitional stage between forward walking and the sideways walking seen in other brachyurans (Chapter 5). These observations point to *L. emarginata* as a good subject to study the transition made by brachyurans from their lobster-like (forward walking) ancestors to the highly successful (sideways walking) crab shape.

### **Aspects of Forward Locomotion Not Addressed in this Study**

#### **Sensory Feedback**

During the present study, I chose to focus on the motor aspect of locomotion, which carried the price of neglecting the important role played by the sensory system in the production of walking behavior. For example, chordotonal organs (Cooper and Hartman, 1999) and muscle receptor organs (Bush and Roberts, 1971; Duysens et al., 2000; Wildman and Cannone, 1996) play a crucial role during walking, as they are involved in reflexes within and between legs. The high degree of coordination between distal and proximal joints in *L. emarginata* may be produced by similar reflex circuits. Future studies of locomotion in spider crabs could benefit from exploring the sensory interactions between these joints, and also between different legs.

#### **Central Output**

One of the great advantages presented by spider crabs is the accessibility of their thoracic nervous system. Because their thorax is not laterally compressed, neurograms from the motor neurons innervating the leg musculature can be obtained from freely walking animals. This type of experiment could provide information about the coupling between neural and muscular activity during naturally behaving animals. At present, these kinds of experiments are impractical in most systems due to technical difficulties.

## **Phylogenetics**

As mentioned previously, data from larval morphology (Rice, 1983) and mitochondrial DNA (Morrison et al., 2002) suggested that majoid crabs belong to a group thought to have first undergone carcinification. The phylogenetic relationships among brachyurans in particular, and crustaceans in general, remain very unexplored at the present time. Future work in this field could allow comparisons between closely related animals with different behavioral repertoires.

## **Behavior Modeling**

A new and exciting approach in neuroethology focuses on creating both virtual and physical behavioral models based on available data (Ayers, 2004; Ritzmann et al., 2004). It would be interesting to integrate the anatomical and kinematic information obtained from this study into a model that sought to explain forward locomotion in spider crabs. The descriptions of the skeletal, muscular and neural systems, as well as the kinematic characterization of forward walking would make spider crabs great subjects for such studies.

## **Muscle Typing**

In Chapter 2, I showed that joints contributing to forward walking have larger ranges of motion in *L. emarginata* than in sideway walking crabs. An implication of this finding is that the same muscle might be used for different jobs in animals that walk sideways or forward. For example, the bender and stretcher muscles that are extensively used by spider crabs during forward walking might be used minimally by sideway walking crabs. Since crustaceans were shown to have different fiber types depending on the task performed by the muscle (McDermott and Stephens, 1988; LaFramboise et al., 2000), it is likely that the fiber type composition of spider crab leg muscles and sideway walking crabs differ in a manner that is consistent with their locomotor preferences (Dewell and Belanger, in preparation). Furthermore, a similar hypothesis could be made for the same muscles of spider crabs, but for legs that are anterior and posterior.

Legs that are anterior in *L. emarginata* pull the COM, while posterior legs push it. This means that the power stroke phase corresponds to opposite motions for anterior and posterior legs.

Investigation of the fiber types composing the muscles of the legs of spider crabs could reveal differential fiber types that reflected the specific task performed by the muscle.

### **Contribution of BM to Locomotion**

The technical challenge of recording kinematics of the joints of two legs while animals were freely walking prevented us from obtaining information about the activity of the basis-merus joint. Although the range of motion for this joint is the smallest, it has several interesting features that make it worthy of study. The BM joint is unique in that its plane of motion is not parallel to the other joints. Contractions of the reductor muscles rotate the long axis of the leg in the anterior direction (Chapter 3). Furthermore, the only intersegmental bundle described for the distal musculature (belonging to the flexor muscle) attaches to the reductor apodeme and could contribute to motion around the BM joint.

During forward locomotion, the BM joint is active during the second half of the power stroke phase (personal observation). Its action rotates the plane of action of MC and PD allowing these joints potentially to generate forward thrust. Without BM activity forward thrust could come only from the joints that articulate in the horizontal plane.

The preceding is by no means an exhaustive list of the possible studies or questions in pedestrian locomotion that might be answered using spider crabs as subjects. As mentioned above, a variety of reasons make spider crabs interesting subjects for neuroethology. Most of the research on crustacean locomotion focused on forward walking macrurans or sideways walking brachyurans. Studies on spider crab locomotion promise to help us bridge the gap between these two groups. The present work provides an anatomical and functional foundation for future studies of legged locomotion in general, and particularly in spider crabs. The answers to many

interesting questions about pedestrian locomotion remain to be learned from this interesting organism.

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Andrés Gabriel Vidal Gadea was born of Elsa Gadea and Roméo Vidal in Montevideo, Uruguay, in 1975. He received his primary and secondary education in Montevideo. In 1993, he had the fortune to receive a scholarship to Lester B. Pearson United World College in Victoria, British Columbia. He graduated with a bilingual International Baccalaureate in 1995. He enrolled as an undergraduate at the University of Victoria in 1997, and graduated with a Bachelor of Science degree in May 2003. He began work toward his doctorate degree in August 2003 in Dr. Jim Belanger's laboratory. He successfully defended his dissertation in April 2008 and will be awarded the degree of Doctor of Philosophy in May 2008.